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MATURITY AND STORAGE STUDIES WITH PEACHES[1]

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INTRODUCTION

The peach production of the Okanagan Valley of British Columbia now exceeds 8,000,000 pounds a year. Over a thousand acres of new plantings have been set out during the past ten years. These plantings consist almost entirely of yellow-fleshed, free-stone varieties such as Elberta, J. H. Hale, Rochester, Vedette, and Valiant. In view of the increasing tonnage of peaches from Okanagan orchards, it is important that accurate information be secured regarding the most desirable harvesting and storage practices.

The stage of maturity at which peaches are picked is of vital importance to *growers*, *packers*, and *consumers* alike. The grower is interested in securing maximum tonnage with minimum harvesting expense. The packer is interested in securing a product which will stand handling and shipment without undue bruising and spoilage. The consumer is interested in securing a high quality product at lowest possible cost.

Most of the peaches grown in the Okanagan Valley are packed in central packing houses handling the fruit from many different growers. Inspection of the fruit delivered to these packing houses has revealed that individual boxes often contain fruits in several stages of maturity. This may be due to carelessness or lack of information on the part of pickers, or it may be due to the grower's desire to cut harvesting cost by picking as much of the crop as possible at one time. On the other hand, there has been a tendency for packing houses to require growers to harvest peaches before they are mature in order to avoid bruising and fill early or special orders.

Various factors enter into the marketing of poor quality peaches, deficient in maturity. However, irrespective of what the causes of low grade peaches on the market may be, the consumer's paramount interest is high quality fruit. There is, therefore, urgent need for a practical maturity test, and for accurate information regarding the relation between such factors as maturity, yield, storage conditions, shipping characteristics and quality.

Peaches may be said to be in storage in the broad sense of the term, from the time they are picked until they are eaten. The normal length of life of this fruit is short, and Okanagan orchards are located at a distance from large consuming markets. Accordingly, packers and shipping organizations, faced with rapidly increasing tonnage, have resorted to precooling and cold storage in an attempt to prolong the marketing season.

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Table 1 shows the periods and temperatures of storage to which Okanagan peaches are ordinarily subjected from the time they are picked until they reach the consumer.

TABLE 1.—TEMPERATURE CONDITIONS EXPERIENCED BY OKANAGAN PEACHES EN ROUTE TO PRAIRIE CONSUMERS

Place	Time	Approximate temperatures
	days	°F.
Stacked in orchard after picking	0 – 1	60 – 100
Held in packing house prior to packing	0 – 1	60 – 80
Held under refrigeration for precooling or storage prior to shipment	1 – 14	32 – 40
In transit in refrigerator car	1 – 5	35 – 45
Held in terminal storage pending distribution	1 – 7	35 – 45
Displayed in retail store	1 – 2	60 – 80
Held by consumer at home	0 – 2	40 – 80
Range	4 – 32	32 – 100

From the information presented in Table 1, it is evident that the time from harvesting to sale on consuming markets extends from a minimum of about 4 days to a maximum of over 4 weeks. As different varieties of peaches are harvested over a period extending from August 1 to September 15, the fruit may be subjected to a wide range of temperatures extending from as low as 40° to as high as 100° F. during the time it is not under refrigeration. During the period of refrigerated storage, temperatures range from 32° to approximately 45° F. While refrigeration for peaches is a recommended procedure under certain conditions, cold stored peaches have not always proved satisfactory, the fruit often being very poor in quality when placed on sale in retail stores. Accurate data are therefore desirable regarding the extent to which the marketing season for peaches can be lengthened by modification of the temperature, and composition of the storage atmosphere.

With these objectives in view, peach harvesting and storage investigations were undertaken at the Summerland Experimental Station. Some of the results secured during the past five years are presented in this report.

REVIEW OF LITERATURE

Peach harvesting, storage and shipping problems have been studied by several investigators in the United States, Canada, Australia and South Africa. Their findings are briefly reviewed in the following paragraphs.

In work conducted with the Elberta variety, McMunn and Dorsey (15) report a gain in volume of 24.4% during the 7 days preceding attainment of optimum picking maturity.

Coe (7) of the Utah Station concluded that in spite of variations, pressure test readings and ground colour were the best indices of maturity for J. H. Hale and Elberta peaches. With fruit from trees of moderate vigour with good exposure to sunlight, and using a pressure tester with a $\frac{5}{16}$ inch point on unpared cheeks of peaches, best picking pressures were

from 12 to 18 pounds and the most satisfactory ground colour was sulphur yellow to amber yellow. Coe noted further that J. H. Hale could be picked in a more advanced stage of maturity than Elberta. Similar conclusions were reached by Morris (16) of the Washington Experiment Station.

Britton and Strachan (6) studied maturity indices for peaches, and by means of shipping tests correlated their findings with the condition of the experimental fruit when it reached the market. They concluded that a combination of the following factors indicates full maturity in peaches: swelling of form, turning of ground colour to yellow, increase in sugar and slight softening of texture.

From the New Jersey Experiment Station have come a series of papers by Blake, Davidson, Addoms and Nightingale (2, 4, 17) dealing with the development and ripening of peaches from the standpoint of physical characteristics, chemical composition, and histological structure of the fruit. These papers distinguish between trees in different conditions of growth; i.e. stunted trees grown under deficient nitrogen supply, and over-vigorous trees heavily fertilized with nitrogen. After the Kraus and Kraybill terminology, these have been termed high carbohydrate and high nitrogen trees respectively. Amongst other things, it was found that firmness of flesh varied inversely as rate of growth until the "shipping-ripe" stage. Between high carbohydrate and high nitrogen trees there were marked differences in rate of ripening, sugar content, firmness, skin olour, pubescence and other characteristics. Fruit from high carbohydrate trees ripened earlier, developed higher colour, was firmer in texture, contained more sugar and could be picked in firmer condition than fruit from high nitrogen trees. The conceptions laid down in these papers, as to causes of variation in fruit from trees of different growth status, offer a satisfactory explanation of the marked variations found in fruit of the same varieties secured from different orchards.

In another paper from the New Jersey Experiment Station, Blake and Davidson (4) suggest the pressure test as the best index of maturity for peaches. They distinguish between soft, firm, and hard ripe fruit. Gauged by a $\frac{5}{16}$-inch pressure tester, peaches suitable for nearby shipping registered 15 to 17, and peaches suitable for long distance shipping, 17 to 20 pounds pressure. They note that a skilled operator should be able to judge a 2-pound pressure difference by eye, but caution that without the use of a flesh testing device he may be misled by the skin colour of some varieties.

In experiments carried out by Harding and Haller (12, 13) it was found that storage of peaches at 36° and 40° F. proved unsatisfactory either because of development of breakdown at these temperatures, or because of rots and decay when subsequently ripened at 70° F. After two weeks' storage at 36° and 40° F., breakdown developed when fruit was removed to a ripening temperature of 70° F. At 32° F. Late Crawford and J. H. Hale held up for 4 weeks and still ripened satisfactorily; Elberta for 3 to 4 weeks; and Belle, Champion and Carman 2 to 3 weeks. In a later publication (11), working with similar varieties, these same investigators caution against holding peaches in cold storage for longer than 2 to 4 weeks, and recommend a storage temperature of 32° F. The breakdown reported

by these investigators as a limiting factor to peach storage, is described in its early stages as presenting a water-soaked appearance around the pit, later developing into larger water-soaked areas followed by flesh browning and mealiness.

From Australia, Adam (1) reports that Catherine Anne peaches held their original condition in shipment better at 32° F. than higher temperatures, and notes that Elberta does not store satisfactorily, quickly becoming mealy and losing flavour. Smith and Willison (18) stored Elbertas at 32°, 37° and 45° F. and found the first temperature most desirable, the fruit keeping satisfactorily from 10 to 14 days.

The seriousness of the mealiness problem in cold stored peaches has been noted especially by the South African industry which annually ships large cargoes of white-fleshed peaches to the English market under refrigeration. Upon arrival on the English market varying percentages of the fruit have been described as "woolly" ("mealy" in American terminology). This led to a study by Davies, Boyes and de Villiers (8, 9) on the relation of storage temperature and delayed cold storage to development of "woolliness". They found that the onset of "woolliness" was greatly accelerated by temperatures of 34° and 37° F. as compared with 32° F. Furthermore they ascertained that a period of delay between harvesting and cold storage controlled "woolliness", a temperature of 75° F. during delay being more effective than 65° or 50° F. With most varieties a period of two to three days at 75° F. was required to give commercial control of "woolliness".

Gas storage of many fruits has attracted attention in recent years. With cherries in particular which have a high carbon dioxide tolerance, Gerhardt and Ryall (10) report that carbon dioxide gas storage along with refrigeration is definitely superior to storage without carbon dioxide. They report improved firmness, brightness, and flavour, and reduced fungal invasion and surface pitting in susceptible fruit. From Australia comes a report (19) that at 34° F. storage life of peaches was increased from 3 weeks to 4 and 6 weeks respectively by storage in atmospheres containing 5 and 10% carbon dioxide, that gas-stored fruit ripened almost as well as that stored in air, but that overstored peaches developed a fermented flavour on ripening. Huelin, Tindale and Trout (14) from Victoria, Australia report that while the average cold storage life of peaches at 32° F. is about 6 weeks, storing in artificial atmospheres containing 8 to 10% carbon dioxide lengthened the storage period by about 10%. Allen and Smock (3) also report that exposure of J. H. Hale peaches for 6 days to 15% carbon dioxide at 45° F. retarded ripening as compared with air-stored checks, and that quality was excellent.

EXPERIMENTAL

Definition of Terms

There is much confusion of terms in the literature describing the maturity and condition of peaches at picking and during storage. When a peach is growing on the tree and approaching the picking stage it is reaching *maturity*; after it is picked it softens and becomes *ripe*. However, medium mature fruit is often described as *hard ripe*, and mature fruit

which has been picked but has not yet ripened is described as *immature.* Fruit has also been described as *maturing in storage.* To help resolve this difficulty and to provide a standardized terminology, the following terms as used in this publication are suggested.

Immature refers to peaches which are not fully developed. They are flat in shape, have a greenish skin and flesh colour, and ripen slowly with poor quality, astringent flavour and shrivelled appearance.

Medium mature refers to peaches which are approaching full development, show greenish-yellow shades of skin colour and dull shades of flesh colour. Such fruit is hard and firm, not quite full size, but will ripen into a fairly good quality product.

Mature refers to peaches which have reached full development on the tree, have filled out in shape, and show yellow shades of skin colour and orange yellow flesh colour. Such fruit is still firm when picked and ripens into a high quality product.

Tree-ripe refers to fruit which has reached an eating-ripe condition on the tree. Such peaches are too soft to pack and are inclined to be stringy and coarse in texture. The quality is inferior to that of peaches ripened after picking.

Unripe applies to the condition of the fruit from the time that it is picked until it softens and becomes ready to eat.

Ripe refers to the fruit during the period that it is in first class eating condition.

Overripe refers to the condition of the fruit when it becomes too soft for sale. In the early stages of overripeness the quality may still be fairly good although the fruit is unsuitable for canning and the skin tends to slough off.

Relations Between Maturity, Size and Shape of Peaches

To secure information regarding changes in size and shape of peaches as they approach maturity, 10 tagged peaches on each of 2 trees of the Rochester, Vedette, and Veteran varieties were measured every few days from August 5 to August 31. Measurements were taken across the suture and also across the cheek. The data secured are presented in Table 2. Asterisks indicate the dates when most of the tagged fruits on the individual trees reached ideal commercial picking condition. The volumes of the fruits have been calculated from their diameters, using the formula for the volume of a sphere, $4/3\pi r^3$. From these data the increase in volume during the week prior to optimum harvesting maturity has been calculated, as shown in Table 2.

From the data presented in Table 2 it will be noted that the shape of Rochester, Vedette and Veteran peaches changed as they approached maturity. With progressing maturity, cheek diameter increased more rapidly than did the diameter across the suture. In other words, the fruits became more rounded. This change in shape of the fruit has proved to be

a reliable maturity index. Table 2 also shows that the increase in volume of the fruit during the week previous to optimum harvesting maturity ranged between 20 and 40%. This is further substantiated by previous work conducted by Britton (6) which showed that with Veteran peaches picked from the same tree on the same day, immature peaches packed 90 to the box, and mature 60 to the box. Similarly, medium mature Elberta peaches packed 65 to the box and mature fruit 54 to the box. It is evident that yield is materially reduced by harvesting peaches in an immature condition.

TABLE 2.—CHANGES IN SIZE AND SHAPE OF PEACHES IN THE PERIOD IMMEDIATELY PRECEDING HARVEST

Date measured 1936	Tree No.	Diameter in inches (average of 10 fruits)					
		Rochester		Vedette		Veteran	
		Suture	Cheek	Suture	Cheek	Suture	Cheek
		in.	in.	in.	in.	in.	in.
Aug. 5	1	2.02	1.97	2.08	1.97	2.04	1.90
	2	1.80	1.69	2.12	2.01	1.92	1.86
Aug. 8	1	2.12	2.09	2.20	2.11	2.12	1.98
	2	1.85	1.78	2.22	2.12	2.00	1.94
Aug. 10	1	2.18	2.17	2.28	2.20	2.21	2.15
	2	1.95	1.88	2.29	2.20	2.07	2.00
Aug. 12	1	2.28	2.26	2.37	2.29	2.28	2.15
	2	2.00	1.93	2.36	2.29	2.12	2.06
Aug. 14	1	2.31	2.33	2.45	2.40	2.35	2.22
	2	2.05	2.01	2.43	2.36	2.17	2.12
Aug. 17	1	2.34	2.38*	2.55	2.54	2.43	2.32
	2	2.14	2.12	2.48	2.47	2.24	2.20
Aug. 19	1	2.39	2.44	2.61	2.58*	2.49	2.39
	2	2.21	2.20	2.55	2.48	2.29	2.26
Aug. 21	1	—	—	2.62	2.58	2.56	2.48
	2	2.28	2.26	2.61	2.57	2.37	2.32
Aug. 24	1	—	—	2.68	2.62	2.65	2.58
	2	2.35	2.32*	2.65	2.62*	2.45	2.42
Aug. 27	1	—	—	—	—	2.71	2.69
	2	2.41	2.43	—	—	2.50	2.49
Aug. 29	1	—	—	—	—	2.73	2.72*
	2	—	—	—	—	2.53	2.52*
Aug. 31	1	—	—	—	—	—	—
	2	—	—	—	—	2.58	2.58
Percentage increase in volume during week prior to optimum harvesting maturity							
	1	27.7		39.1		26.6	
	2	33.2		20.5		23.4	

Average increase in volume during week prior to optimum harvesting date—28.4%.

* Date of optimum maturity.

Relations Between Soluble Solids, Flesh Colour, Skin Colour and Hardness

To study the relations between soluble solids, flesh colour, skin colour, and hardness in maturing peaches, samples in various stages of maturity were gathered from the same tree on the same day. These fruits were sorted into three grades; immature, medium mature, and mature, as defined earlier in this report. From 10 fruits in each grade juice was expressed and the soluble solids determined by use of a Zeiss refractometer. Firmness was measured by punching the unpared cheeks with a $\frac{7}{16}$ inch point Ballauf pressure tester. This procedure was followed with the Rochester, Vedette, Veteran, Valiant, J. H. Hale and Elberta varieties. Data representative of the results secured are embodied in Table 3.

The data presented in Table 3 indicate that the soluble solids content increased in all varieties tested as they approached maturity. Within each variety the most mature fruit invariably had the highest soluble solids content. However, the differences in soluble solids between mature and immature fruits were small. Furthermore the soluble solids content of mature fruit differed somewhat with variety.

Flesh colour was greenish yellow in immature fruits to orange in mature fruits. Almost without exception, peaches of the 6 varieties tested had a light orange yellow flesh colour while still in sufficiently firm condition to ship satisfactorily. The flesh of less mature fruit had a distinct greenish cast.

With most varieties the skin on the unblushed side of the fruit was light green in immature fruit and pale yellow in mature fruit. However the Rochester variety was characterized by a reddish blush over the entire fruit even when immature.

TABLE 3.—INFLUENCE OF MATURITY ON SKIN COLOUR, FLESH COLOUR, HARDNESS, AND SOLUBLE SOLIDS CONTENT

Variety	Date picked	Stage of maturity	Skin colour	Flesh colour	Pressure test	Soluble solids
					lb.	%
Rochester	Aug. 15	Mature	Blush all over	Orange yellow	22.2	12.0
		Medium mature	Blush all over	Orange yellow calyx end, light green stem end.	26.9	11.3
		Immature	Blush all over	Yellow green	27.2	11.1
Vedette	Aug. 19	Mature	Pale yellow	Orange yellow	21.3	11.8
		Medium mature	Greenish yellow	Light orange yellow, green around pit.	27.1	11.0
		Immature	Light green	Mostly greenish	29.0	10.2
Veteran	Aug. 25	Mature	Pale yellow	Orange	20.7	12.0
		Immature	Light green	Greenish yellow	29.2	10.7
Valiant	Aug. 25	Mature	Pale yellow	Orange yellow	20.0	13.8
		Immature	Light green	Greenish yellow	27.1	11.8
J. H. Hale	Sept. 10	Mature	Pale yellow to orange.	Yellow to bright orange.	18.5	14.3
		Immature	Light green	Pale green, orange at pit.	25.7	12.4
Elberta	Sept. 12	Mature	Citron yellow	Pale orange yellow	21.8	13.3
		Immature	Greenish yellow	Greenish yellow	28.8	12.2

With regard to hardness, the data presented in Table 3 show that mature fruit had a consistently lower pressure test than immature fruit. As a general rule, fruits testing about 20 pounds with a $\frac{7}{16}$ inch pressure tester were firm enough for fresh shipment and developed excellent quality, whereas fruit testing over 25 pounds failed to attain good dessert quality. However, a considerable variability in firmness was noted between fruits in the same sample, and even between two cheeks of the same peach.

Influence of Storage Temperature on Peaches

During the past four years peaches have been stored at 32°, 40°, and 65° F., and removed at regular intervals to a ripening room held at 65° F. and 85 relative humidity. The fruit used in the experiments was picked at two stages of maturity, "mature" meaning fruit that was fully developed yet sufficiently firm in texture to be in ideal shipping condition, and "medium mature" meaning fruit that was fairly good in quality but two days or so less mature than the first lot. The fruit was placed under refrigeration the same day it was picked. At approximate weekly intervals, samples of peaches were removed from cold storage to a 65° F. ripening room where notes were taken on quality, diseases, and length of storage life. The data in Table 4 show the number of days peaches were held satisfactorily under refrigeration at 32° and 40° F.

In the studies reported in Table 4 the limiting factor to cold storage of peaches was a low temperature type of breakdown which has been described as mealiness. South African investigators (8, 9) speak of it as "woolliness". The disorder is characterized by a dry spongy condition

Table 4.—Cold storage life of peaches prior to onset of mealiness at 32° and 40° F.

(fruit stored within 6 hours of picking)

Variety	Maturity	Cold storage life in days									
		32° F.					40° F.				
		1935	1936	1938	1939	Ave.	1935	1936	1938	1939	Ave.
Golden Jubilee	Mature	—	—	—	22	22	—	—	—	15	15
Rochester	Mature	23	21	14	22	20	9	7	14	14	11
	Medium	23	14	—	22	20	9	7	—	7	8
Vedette	Mature	16	17	14	22	17	9	10	7	14	10
	Medium	16	17	—	14	16	9	10	—	7	9
Valiant	Mature	—	14	14	16	15	—	11	7	23	14
	Medium	—	14	14	16	15	—	11	7	16	11
Veteran	Mature	10	14	—	22	15	10	11	7	8	9
	Medium	10	14	14	16	13	10	11	7	8	9
J. H. Hale	Mature	—	10	14	14	13	—	7	7	7	7
	Medium	—	10	—	14	12	—	0–7	—	7	7
Elberta	Mature	—	10	14	—	12	—	7	7	—	7
	Medium	—	10	—	—	10	—	7	—	—	7

of the flesh which does not improve even after the fruit has been exposed to ripening temperature. The most deceptive aspect of this disease is the fact that to all outward appearances affected fruit is normal and attractive. Its true internal condition only becomes apparent upon cutting or biting into the flesh.

It will be observed that for most varieties, storage at 40° F. was limited to 1 to 2 weeks, and at 32° F. to 2 to 3 weeks. Fruit could usually be held at least one week longer at 32° F. than at 40° F. In Table 4 the varieties have been arranged in order of ripening, Golden Jubilee first and Elberta last. It is interesting to note that the earlier maturing varieties withstood a longer period of cold storage without developing mealiness than did the later ripening sorts, such as J. H. Hale and Elberta.

Similar samples to those stored at 32° F. and 40° F. were held at 65° F. The length of time the fruit held at 65° F. remained in good eating condition is shown in Table 5.

It will be noted that with mid-season varieties there was an increase in storage life of several days with medium mature as compared with mature fruit, while with J. H. Hale and Elberta which mature later, storage life was nearly doubled in the medium mature samples. Both mature and medium mature samples of J. H. Hale and Elberta actually stored longer in good condition at a continuous temperature of around 65° F. than those which were stored immediately at 32° or 40° F. for a week or more before being ripened at 65° F.

It is a matter of importance to know how long cold storage peaches require to ripen, and how long they remain in marketable condition after removal from refrigeration. Data relating to this problem are found in

TABLE 5.—STORAGE LIFE OF PEACHES HELD CONTINUOUSLY AT 65° F. FROM TIME OF PICKING (AVERAGE 1935–36–38–39)

Variety	Maturity	Days to ripen at 65° F.	Days from ripeness to overripeness at 65° F.	Total storage life in days
Golden Jubilee	Mature	2.0	14.0*	16.0
Rochester	Mature	4.0	6.5	10.5
	Medium	4.0	9.0	13.0
Vedette	Mature	2.5	7.5	10.0
	Medium	4.5	10.0	14.5
Valiant	Mature	4.0	14.0	18.0
	Medium	5.0	13.0	18.0
Veteran	Mature	3.0	11.0	14.0
	Medium	4.5	11.0	15.5
J. H. Hale	Mature	4.0	15.0	19.0
	Medium	11.0	20.0	31.0
Elberta	Mature	4.0	12.0	11.0
	Medium	9.0	22.0*	31.0

* One year's results only.

Table 6. The figures represent an average of the results secured over a period of 4 years. Data are presented only for fruit cold stored for 1 and 2 weeks, for almost invariably it was found that cold storage for 3 weeks or more resulted in the development of mealiness.

TABLE 6.—INFLUENCE OF LENGTH AND TEMPERATURE OF COLD STORAGE ON TIME REQUIRED FOR RIPENING, AND PERIOD OF EDIBILITY OF PEACHES AT 65° F. (AVERAGE 1936–38–39)

Variety	Temper-ature °F.	Cold stored one week		Cold stored two weeks	
		Days to ripen	Days from ripeness to overripeness	Days to ripen	Days from ripeness to overripeness
Golden Jubilee	40	7	14	3	8
	32	7	14	3	8
Rochester	40	1	4.5	1	4
	32	2	4	2	4.5
Vedette	40	1	7	0	5
	32	2	8	2	6
Valiant	40	3	8	2	6
	32	3	8	3	6.5
Veteran	40	3	6	2	5
	32	4	8	2	5
J. H. Hale	40	4	8	Mealy	Mealy
	32	4.5	11	4	6
Elberta	40	3	5	Mealy	Mealy
	32	3	8	Mealy	Mealy

It will be noted that in most cases there was only a slight reduction in storage capacity at 40° as compared with 32° F. but that with 2 weeks' as compared with 1 week's cold storage there was a distinct loss in subsequent storage life; 2 weeks' as compared with 1 week's cold storage decreased subsequent life of the fruit at 65° F. by at least one-third.

Influence of Delayed Cold Storage on Peaches

To ascertain the effect on Okanagan grown peaches of a delay at comparatively high temperatures before cold storage, an experiment was conducted in 1939. Six varieties of yellow-fleshed peaches were studied in two stages of maturity: mature, and medium mature. At picking, the fruit was divided into 3 lots, one cold stored within 6 hours, one delayed for 1 day at approximately 75° F., and one delayed for 2 days at 75° F. before being placed under 32° F. and 40° F. refrigeration. At weekly intervals samples of each lot of fruit were removed to 65° F. for ripening and examination. By systematic removals of fruit from storage the length of time the fruit could be cold stored and subsequently ripened without development of mealiness was determined. Data concerning the "mealiness-free" storage life of peaches are presented in Table 7.

It will be noted that with all varieties except J. H. Hale, a delay at high temperature before storage at 32° F. markedly increased storage life. In most cases 2 days' delay was more effective than 1 day's delay; 2 days' delay increased storage life about 50% as compared with immediate storage. In fruit stored at 40° F. the beneficial effect of delayed storage was more marked (except with the Valiant variety) than with fruit stored at 32° F. In several cases the "mealiness-free" storage life was doubled by a delay at high temperature prior to cold storage. It is also interesting that 1 day's delay had practically the same effect as 2 days' delay in controlling mealiness of fruit stored at 40° F. In general the less mature the fruit was at harvest, the greater was the period of delay required to prevent mealiness.

Table 7.—Effect of delayed cold storage on "mealiness-free" storage life of peaches at 32° F. and 40° F.

Variety	Maturity	Cold storage life in days					
		32° F.			40° F.		
		Stored at once	1 day delay	2 day delay	Stored at once	1 day delay	2 day delay
Golden Jubilee	Mature	22	30	30	15	30	30
Rochester	Mature	22	30	30	11	30	30
	Medium	22	22	30	8	30	30
Vedette	Mature	22	28	35	10	22	28
	Medium	14	22	35	9	14	28
Valiant	Mature	16	23	30	14	8	16
	Medium	16	16	30	11	8	8
Veteran	Mature	22	22	29	9	22	22
	Medium	16	29	22	9	22	22
J. H. Hale	Mature	7	7	7	7	7	7
	Medium	7	7	14	7	14	7
Average		17	21.5	26.5	10	19	21

The effect of delayed storage on the edible life of peaches after removal to a ripening room is depicted in Table 8.

From the data embodied in Table 8 it is apparent that delayed storage resulted in no appreciable decrease in the edible life of the fruit. Life of fruit stored at 40° F. was only slightly less than that of fruit stored at 32° F. It should be noted here, however, that the longer the fruit was held in cold storage the faster was the rate at which it ripened. Nevertheless, even after 2 weeks' cold storage, fruit previously subjected to 1 and 2 days' delay at 75° F. remained in firm condition with good flavour for at least a week after removal to a 65° F. ripening room.

The quality of all lots of fruit prior to the onset of mealiness was usually excellent. Particularly noticeable was the improvement in quality of delayed storage fruit over immediate stored fruit even when no mealiness had developed in the latter. Delayed storage fruit was usually finer textured, juicier, and of better flavour than immediate stored fruit. Only on the very latest removals of delayed storage fruit was there any noticeable loss of flavour, even though mealiness was not present.

Table 8.—Life of delayed cold storage peaches after removal to ripening room at 65° F. following two weeks' at low temperatures

Variety	Maturity	Edible life in days at 65° F.					
		32° F.			40° F.		
		Stored at once	1 day delay	2 day delay	Stored at once	1 day delay	2 day delay
Golden Jubilee	Mature	8	8	8	8	8	8
Rochester	Mature	8	7	7	6	6	4
	Medium	10	11	9	10	9	9
Vedette	Mature	6	8	7	6	5	6
	Medium	6	8	8	6	6	9
Valiant	Mature	11	9	9	8	7	8
	Medium	13	13	12	13	13	13
Veteran	Mature	11	11	12	17	7	8
	Medium	14	14	14	Mealy	7	6
J. H. Hale (after 1 week at low temperature)	Mature	12	12	12	17	9	12
	Medium	20	13	13	17	12	12

Influence of Carbon Dioxide Gas on Peaches

Two carbon-dioxide gas storage experiments were carried out with peaches in 1938, one an initial treatment for 2 and 4 days in high carbon dioxide concentration and the other, continuous storage of peaches under controlled ventilation.

The high initial carbon dioxide treatments of peaches were carried out in 4-gallon containers at a temperature of 65° F. for 2 and 4 days respectively. The gas used was supplied from a compressed cylinder and concentrations in the containers were measured with an Orsat apparatus. At the end of the treatment the fruit was removed to air and compared with similar air-stored fruit held at the same temperature. Three varieties were used, Vedette, Veteran, and Valiant, all picked in mature condition. The data secured are given in Table 9.

The data shown in Table 9 indicate that concentrations of approximately 30% carbon dioxide for short periods did not result in any increase in total storage life, although the rate of softening was greatly slowed down while the fruit was in the gas. Two days' carbon dioxide treatment did not harm the flavour which the fruit developed on ripening, but fruit exposed to carbon dioxide for four days developed an acid alcoholic flavour on ripening. In addition, the Veteran samples both developed a watery type of flesh breakdown in 25% of the fruits.

The second experiment undertaken involved holding peaches under 7.5% carbon dioxide at 40° F. in a ventilated galvanized iron container for 1- and 2-week periods. The fruit was then removed to air at 65° F. and compared as to qualify and storage life with fruit held for the same length of time in air at 40° and 32° F. Three varieties of peaches were used for the experiment, Vedette, Rochester, and Valiant. The fruit was picked in the mature condition. The results are summarized in Table 10.

TABLE 9.—EFFECT OF HIGH INITIAL CONCENTRATIONS OF CARBON DIOXIDE ON PROLONGING STORAGE LIFE OF PEACHES AT 65° F.

Variety	Days in high CO_2	Per cent CO_2	Condition on removal	Quality when ripe	Days to become overripe at 65° F.
		%			
Vedette	2	23	Firm and unripe	Good	17
	4	26	Firm and unripe	Fair, sour, alcoholic flavour	17
	Air	0	Eating ripe	Good	17
Veteran	2	30	Firm and unripe	Good, 25% watery breakdown	12
	4	37	Firm and unripe	Fair to poor, alcoholic, 25% watery breakdown.	12
	Air	0	Eating ripe	Good	11
Valiant	2	35	Firm and unripe	Good	18
	4	29	Firm and unripe	Poor, acid, alcoholic flavour	16
	Air	0	Eating ripe	Good	18

TABLE 10.—EFFECT OF STORAGE OF PEACHES IN 7.5 PER CENT CO_2 AT 40° F., AS COMPARED WITH STORAGE IN AIR AT 40° F. AND 32° F.

Variety	Storage treatment before removal to 65° F. ripening room			Appearance when ripe	Quality when ripe	Days to become overripe from picking date
	Temp.	CO_2	No. of days			
	°F.	%				
Vedette	40	7.5	7	Good	Fair	20
	40	0.0	7	Good	Good	20
	32	0.0	7	Good	Good	22
	40	7.5	14	Good	Fair	24
	40	0.0	14	Good	Fair, slightly mealy	22
	32	0.0	14	Good	Good	28
Rochester	40	7.5	7	Good	Good	16
	40	0.0	7	Good	Good	12
	32	0.0	7	Good	Good	14
	40	7.5	14	Good	Fair, slightly mealy	19
	40	0.0	14	Good	Fair	18
	32	0.0	14	Good	Good	18
Valiant	40	7.5	7	Good	Fair to good	16
	40	0.0	7	Good	Good	18
	32	0.0	7	Good	Good	21
	40	7.5	14	Good	Mealy, no good	Over
	40	0.0	14	Good	Good, slightly mealy	18
	32	0.0	14	Good	Good	28

The data secured indicate that storage of peaches in 7.5% carbon dioxide at 40° F. increased the life of the fruit after removal to air by a few days in several instances. However, in almost every case the fruit was of poorer quality than that stored in air at 40° F. With Valiant and

Rochester, gas storage favoured development of mealiness. Gas-stored fruit after ripening in air took on a rather disagreeable acid flavour which persisted as long as the fruit was in eating condition. Furthermore, comparable peaches stored in air at 32° F. had a longer life than the fruit stored in 7.5% carbon dioxide at 40° F.

DISCUSSION

The gain in volume of from 20 to 39% during the week prior to harvesting maturity reported in this paper is in substantial agreement with the findings of McMunn and Dorsey (15). From these results it is evident that premature picking involves a heavy loss in tonnage.

In determining the optimum picking maturity of the Golden Jubilee, Rochester, Vedette, Valiant, J. H. Hale, and Elberta varieties, the pressure test recommended by Coe (7), Morris (16), and Blake and Davidson (4) was found to have value as a maturity test, but its practical application is limited by the fact that peaches ripen unevenly. The skin colour test recommended by Coe (7) was found to be a useful guide to maturity with all varieties investigated except Rochester, of which the skin is almost completely blushed prior to optimum picking maturity. The results reported in this paper indicate that flesh colour and fruit shape are reliable indices of maturity for the above varieties when grown under Okanagan conditions. In judging the picking maturity of peaches, external indications such as skin colour and fruit shape must necessarily be the guiding factors in differentiating mature from immature fruit, but these factors can be correlated with firmness and flesh colour as a final check.

Data presented in this paper indicate that onset of mealiness limits the time that yellow-fleshed free-stone peaches grown in the Okanagan can be held under cold storage conditions. In accord with the work of Adam (1), Harding and Haller (12, 13), and Willison (18), results indicate that peaches can be held longer at 32° than at 40° F. before mealiness develops, and that even under the best of conditions the fruit should not be held in cold storage for more than 3 weeks. Similar to the findings of Davies, Boyes and deVilliers (8, 9), a delay of two days at 75° F. before cold storage materially delayed the onset of mealiness and improved the quality of the fruit.

Okanagan grown peaches failed to show the favourable response to gas storage which has been reported with the Australian product (14, 19).

RECOMMENDATIONS

In formulating recommendations for harvesting and storing the Okanagan peach crop consideration must be given to the fact that the major consuming markets are located several hundred miles from the producing area. The facilities available for harvesting, packing and shipping the crop in a comparatively short period of time must also be taken into account. Furthermore the grower's interest in low production costs, the packer's demand for firm fruit which will withstand commercial handling without wastage, and the consumer's desire for a high quality product are somewhat incompatible, with the result that practical recommendations must involve compromise.

However, the results of the investigations reported in this paper together with general observations made in orchards, packing houses, and consuming markets indicate that, given the intelligent co-operation of the various parties involved, the Okanagan peach crop can be handled to the satisfaction of all concerned. With this aim in view the following recommendations are made to growers, packers, dealers and consumers.

To Growers

As the peach crop ripens unevenly, it is necessary to make several pickings to ensure harvesting of the individual fruits at the correct stage of maturity. This is especially true of varieties such as Rochester, which mature over a comparatively long season, often necessitating as many as 5 pickings. However even with varieties like Elberta which ripen their crop more uniformly, 3 pickings are usually required. The peach picker should learn to recognize at a glance the small differences in shape and skin colour which distinguish a mature from a medium mature peach. His aim should be to harvest each individual fruit at the mature stage when it has filled out in shape, shows yellow shades of skin colour, orange-yellow flesh colour and is still firm enough to stand commercial handling.

In the actual operation of picking, the fruit should be grasped in the palm of the hand, care being taken to avoid bruising by pressure with the fingers. It is advisable to pick into metal buckets rather than canvas picking bags. For transporting the fruit from orchard to packing house, boxes of solid construction are advisable. If apple boxes are used they should be filled only two-thirds full and lined with newspaper to prevent entry of dirt and to minimize bruising caused by the flexible bottom.

To Packers

Even when picked in ideal condition for shipment peaches require more careful handling than apples. Packing house operators should bear this fact in mind and take every practical precaution possible to prevent bruising during the grading and packing operations. The less mature fruit should be shipped to more distant markets. In this connection grading for size usually grades for maturity as well, the small sizes being less mature than the large. Consideration should be given to the feasibility of packing very large sizes in single layer flats.

A special effort should be made to move peaches rapidly into consumption without resort to cold storage. Peaches do not respond well to cold storage temperatures which tend to induce a mealiness type of breakdown. This is especially true of the Elberta and J. H. Hale varieties which actually remain in good condition longer when held at 60° F. to 70° F. than when subjected to cold storage temperatures. Earlier varieties such as Rochester and Veteran may safely be stored at 32° F. for 3 weeks or at 40° F. for 2 weeks provided they are subjected to delayed storage at about 75° F. for 2 days before being placed in cold storage.

To Retail Dealers and Consumers

It is seldom that recommendations are extended to fruit dealers and consumers remote from the source of supply. However in order that the consumer may receive the highest quality product possible it is important that dealers be familiar with the storage and ripening requirements of peaches. As peaches do not keep well in cold storage each variety should

be moved into consumption as rapidly as possible. When early varieties such as Vedette are stored into the Elberta season, mealiness, wastage and loss of quality are sure to result. Both dealers and consumers should be fully acquainted with the fact that peaches ripen most satisfactorily in a warm moist atmosphere.

SUMMARY

Attention is drawn to the rapid increase in production of yellow-fleshed free-stone peaches in the Okanagan Valley of British Columbia. The urgent need for reliable data on desirable harvesting and storage procedure is stressed. The literature on these subjects is briefly reviewed and maturity terms are defined. Experiments designed to ascertain the influence of maturity on size, hardness, skin colour, flesh colour, soluble solids content, and quality of peaches are described and the results presented. Storage experiments conducted to secure information regarding the influence of storage temperature, delayed storage, and gas storage on the life of peaches are outlined and the results stated. The conformity of these results with those reported in the literature is briefly discussed.

The most important findings are:

1. During the week immediately preceding optimum harvesting maturity, peaches increased in volume approximately 4% per day.

2. Fruit shape and skin colour associated with flesh colour and firmness were reliable indices of maturity. As a general rule peaches of satisfactory maturity for shipment as fresh fruit were rounded in shape, showed yellow shades of skin colour, orange-yellow flesh colour and a firmness of about 20 pounds as measured by the Ballauf pressure tester, using a $\frac{7}{16}$ inch point.

3. Increase in soluble solids as measured by the Zeiss refractometer was not a satisfactory maturity index because the range between mature and immature fruit was too narrow.

4. The cold storage life of peaches was limited by development of a "mealiness" type of breakdown. Early varieties developed this disorder slower than later varieties. In most cases mealiness became evident in fruit removed to a 65° F. ripening room after 1 to 2 weeks' storage at 40° F., and after 2 to 3 weeks' storage at 32° F.

5. With most varieties, development of mealiness was greatly retarded by a delay at 75° F. before placing in cold storage; 2 days' de ay was more effective than 1 day's delay.

6. Life of peaches subsequent to removal from cold storage to 65° F. was not appreciably diminished by 1 or 2 days' delayed storage at 75° F.

7. Quality, texture, and juiciness were improved in all varieties by delayed cold storage as compared with immediate cold storage.

8. After removal from cold storage to a ripening temperature at 65° F., fruit which had been stored at 32° F. remained in good condition only a slightly longer time than fruit which had been held at 40° F. Quality in both cases was good provided the storage period was short.

9. Peaches held at 65° F. continuously from time of picking remained in good condition from 10 to 19 days for mature, and from 13 to 31 days for medium mature fruit.

10. Storage of peaches in atmospheres containing carbon dioxide gave discouraging results.

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INVESTIGATIONS CONCERNING THE COUMARIN CONTENT OF SWEET CLOVER[1]

I. The Breeding of a Low-Coumarin Line of Sweet Clover—*Melilotus alba*

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The importance of biennial sweet clover as a forage and soil improving crop in Western Canada has led to the expenditure of considerable effort towards the production of improved strains and varieties of the more widely grown species. At the Dominion Forage Crops Laboratory, Saskatoon, Sask., considerable attention has been given, during recent years, to the production of coumarin-free varieties or at least varieties in which the coumarin content is comparatively low.

Coumarin is the substance which is largely responsible for the stinging, bitter taste of ordinary sweet clover. It is frequently referred to in literature as the "bitter principle". In addition to rendering the plant unpalatable to animals which are unaccustomed to the flavour of coumarin, it has been shown to be indirectly responsible for the so-called "sweet clover disease of cattle" which manifests itself in delayed clotting of the blood, and under certain conditions may result in the death of the animals.

Another more recent condition, attributed to the coumarin content of sweet clover, is the so-called "Melilot-taint" of wheat which has become troublesome, and has resulted in considerable loss to wheat growers in certain areas of Western Canada (17).

It is probable therefore that a low-coumarin or a coumarin-free variety of sweet clover would go a long way towards eliminating those conditions which have at certain times raised serious objections to the use of this otherwise valuable legume.

Smith and Brink (11) definitely established the fact that the development of the toxic principle in poorly cured sweet clover hay depended upon the presence of coumarin. Quick (8) reported that in experiments with rabbits the inclusion of as much as 5% of dried alfalfa meal with toxic sweet clover hay prevented the development of the disease. Smith (10) found that the inclusion of as much as 12.9% of alfalfa with toxic sweet clover hay failed to prevent the development of the disease. The committee on the Melilot taint of wheat (17) reported a definite tainting of wheat when it is grown, harvested and threshed in combination with sweet clover plants. They have found also that this Melilot taint persists in bread made from such wheat. This condition resulted in the grading of a number of carloads of wheat "rejected" in 1938 and 1939 with a considerable loss to the grower.

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Obermayer (6) pointed out the possibilities of sweet clover for fodder production on poor soils if it were not for the bitter taste and peculiar odour of the plant. He was the first to develop a method for the determination of the coumarin content in sweet clover. However, his method is subject to errors, and is so slow that it is of little value where a large number of determinations must be made in a short time.

Kirk (5) referred to the testing of 8 different types of sweet clover for coumarin content. He noted little variation in the amount of coumarin contained in them. Ufer (15, 16) announced the development of a rapid test, and reports the testing of over 900,000 sweet clover plants. He selected 51 plants, 3 of which he classified as coumarin-free, but these died without producing progeny. Suvorov (13) reported that after three years' work he had obtained sweet clover plants with coumarin contents of 0.064 to 0.056%. Two years later (14) he announced that he had selections containing as low as 0.033%. Gelcinskaja and Bordunova (4) reported variations in coumarin content of from 1.0 to 0.026% in white blossom sweet clover and 0.86 to 0.062% in yellow blossom sweet clover. Pokrovski (7) reported finding 114 lucerne-like sweet clover plants having insignificant amounts of coumarin. However, subsequent tests convinced him that the low-coumarin content was linked with lack of winter hardiness. Details of methods used by these investigators in determining the coumarin content of sweet clover have not been made available. Dworak (3) has reported a technique of selection based upon odour.

Clayton and Larmour (2) developed a colorimetric method for determining the coumarin and melilotic acid content of sweet clover. Although their method, because of interfering plant pigments and the presence of phenolic compounds in the plant extract, cannot be regarded as strictly quantitative, it provides a practical, comparative test for the plant breeder. It has the important advantage of being rapid.

MATERIALS AND METHODS

With slight modifications the procedure used in testing was the same as that described by Stevenson and Clayton (12). In extracting from the foliage, 1 gram of fresh green leaves was weighed out, and this was ground in a mortar and pestle by adding about 1 cc. of washed sharp sand and moistening with 50% methyl alcohol. Using 50% methyl alcohol, the whole was then washed into a test tube calibrated at the 51 cc. mark. The tube was then filled almost to the 51 cc. calibration with 50% methyl alcohol and fitted with a rubber stopper and a 12-inch glass tube which served as an air condenser. The tube was immersed in a bath at 85° C. for 15 minutes. After heating, the inside of the condenser was washed down and the volume made up to 51 cc. with the 50% methyl alcohol. After shaking for 1 hour on a rotary shaker the extract was ready for testing.

In extracting from the seed the procedure was modified in the fall of 1937 to provide for an incubation period, as suggested by Roberts and Link (9). With the modified extraction method ½ gram of seed was finely ground in a mortar and pestle and then placed in a test tube, 25 cc. of water added, and the tube incubated in a water bath at 40° C. for 1 hour. Then 25 cc. of methyl alcohol were added and the tube shaken for 1 hour on the rotary shaker.

The procedures used in testing extracts from leaf and seed material were identical and were the same as described by Stevenson and Clayton (12).

Sampling was accomplished by stripping leaves along a branch, care being taken not to sample when the leaves were wet from dew or rain. When testing was done on the first year's growth the plants were usually sampled in late August or early September, while on the second year's growth the sampling was done in the late bud to early flowering stage.

Since the beginning of the project in the winter of 1933–34 approximately 7000 tests have been made on vegetative material and 3000 tests on seed. In this paper it is proposed to follow the progress of only those selections made after the first testing of seed, one of which gave rise to the low coumarin strain. The original selections and the selections in the next generation were based upon tests of the seed. In all subsequent selections only the coumarin content of the vegetative material was considered.

The general type of procedure followed was to sow selfed seed in greenhouse flats in March, and to transplant to the field in early June. In the nursery the plants were grown in progeny rows, the plants being spaced 3 feet apart in the rows, and the rows separated by 3 feet. With the exception of the first generation of selfing the plants were tested in the fall of the year they were set out. Following this test any plants which appeared outstanding were dug up and brought into the greenhouse. During the winter the plants were tested at the flowering stage for coumarin content, and selfed seed was obtained.

Throughout this paper coumarin content is expressed as a percentage of green weight of the sample for both vegetative and seed material. It was considered that errors introduced through neglecting variations in dry matter content would be well within the limits of accuracy of the test itself. If it is desired to make an approximate comparison between the amount of coumarin in the seed and vegetative material, it is necessary to multiply the latter by about 4.5.

PROGRESS IN THE SELECTION OF LOW-COUMARIN STRAINS

Stevenson and Clayton (12) have reported the initial steps in the breeding program with which this paper is concerned. This early work will be recapitulated here for the sake of continuity.

In the winter of 1933–34 open fertilized seed from single plants of Arctic sweet clover was tested for coumarin content. Nine plants were selected as having unusual amounts of coumarin. Five of these individuals were found to contain between 0.10 and 0.15% coumarin, and four of them had over 1.00% of the substance in the seed. Open fertilized progenies of these plants were set out in the spring of 1934. No tests were made on the foliage of these progenies but they were selfed in 1935. Open fertilized seed was also collected from them for testing during the following winter. In Table 1, which is taken from Stevenson and Clayton's Table V, the results of this testing are shown.

These results showed that a marked difference existed between the progenies of the high and of the low selections, thus indicating that the differences had a genetic basis. Based upon this test 11 plants, 10 of which had 0.15 and one 0.20%, were taken as selections for low coumarin.

To determine whether or not the coumarin content could be increased by selection, 5 individuals which contained from 1.05 to 1.40% were also selected in 1936.

In the fall of 1936 the foliage of the first year's growth of the progenies from the above selections was tested. The results are presented in Table 2 in the form of a frequency distribution by progenies.

TABLE 1.—COUMARIN CONTENT OF SEED OF PROGENIES FROM SWEET CLOVER PLANTS SELECTED FOR HIGH AND LOW COUMARIN CONTENTS

Selection No.	Test of parent	Classes in percentage								
		0.15	0.20	0.30	0.40	0.50	0.60	0.70	0.80	0.90
S-34-35	.15		12	50	20	1				
S-34-36	.15	1	15	32	25	5	4			
S-34-37	.15	11	26	24	7	2	1			
S-34-38	.15		5	17	12	7	2			
S-34-39	.15	3	11	21	3					
S-34-40	1.00+						1	2	6	4
S-34-41	1.00+					1		2	2	
S-34-42	1.00+							1	1	2
S-34-43	1.00+				2	10	5	8	3	3

		Classes in percentage						
		1.00	1.05	1.10	1.20	1.30	1.40	Ave. test.
S-34-35	.15							.330
S-34-36	.15							.337
S-34-37	.15							.260
S-34-38	.15							.363
S-34-39	.15							.267
S-34-40	1.00+	14	4	3	6	3		1.000
S-34-41	1.00+	4	1	3	14	5		1.098
S-34-42	1.00+	2	3	5	11	6	2	1.439
S-34-43	1.00+	1	1					.653

TABLE 2.—THE FREQUENCIES OF SWEET CLOVER PLANTS IN CLASSES OF COUMARIN PERCENTAGE. THE PLANTS WERE TESTED IN 1936 IN THE FALL OF THEIR FIRST YEAR OF GROWTH, AND WERE PROGENIES OF HIGH AND LOW COUMARIN PARENTS

Strain No.	Seed test of parent	Classes of coumarin percentage											Ave.
		0.00	0.01–0.04	0.05–0.09	0.10–0.14	0.15–0.19	0.20–0.24	0.25–0.29	0.30–0.34	0.35–0.39	0.40–0.44	0.45–+	
S-34-35-1	.15					–	6	8	6	10	9	1	.334
S-34-36-1	.15					4	8	8	5	5	5	5	.312
S-34-36-2	.15					5	13		2	8	8	3	.310
S-34-36-3	.15			2	6	8	2	2	9	3	5	2	.262
S-34-37-1	.15			4	4	5	12	1	4	7	2	1	.245
S-34-37-2	.15			6	5	9	9	7	2	2			.195
S-34-37-3	.15			1	6	16	7	3	2	4		1	.211
S-34-37-4	.15			1	7	12	5	3	6	2	1		.215
S-34-38-1	.20	1	5	5	8	13	2	3	1	2			.149
S-34-39-1	.15	7	1	1	4	7	7	6	4	3			.186
S-34-39-2	.15			1	1	15	10	3	4	4	2		.236
S-34-40-1	1.20				6	9	4	8	5	7	1		.248
S-34-41-1	1.25			2	6	13	4	11	3	1			.206
S-34-41-2	1.40			1	5	13	2	3	9	4	3		.244
S-34-41-3	1.30			1	13	7	7	8	2	2			.246
S-34-42-1	1.05		2	5	3	6	5	11		5	3		.223

The most interesting and encouraging result of this testing was to find a few plants which would give no test for coumarin. The tests on these plants were checked and verified. As shown in Table 2, 7 out of 40 plants of strain S-34-39-1 gave a negative test. One such plant appeared in strain S-34-38-1 which also contained 4 other low testing plants. Based upon these tests, 11 selections were dug up and brought into the greenhouse. These included 4 of the plants from line S-34-39-1 which tested 0.00%, 4 plants from S-34-38-1 which tested 0.04, 0.05, 0.05, and 0.08%, 1 plant from each of lines S-34-37-3, and -4 which had been selected for feeding by rabbits, and one plant which tested 0.04% from S-34-42-1, a high coumarin line. Selfed seed of these selections was obtained during the winter.

Progenies of the above selections were planted in the spring of 1937, and coumarin tests of the foliage made in the fall. The result of this testing is shown in Table 3.

Table 3.—The frequencies of sweet clover plants in classes of coumarin percentages. The plants were tested in 1937 in the fall of their first year of growth and were progenies of high and low coumarin parents

Strain No.	Test of parent		Classes of coumarin percentage										Ave.
	Foliage	Seed	0.00	0.01–0.04	0.05–0.09	0.10–0.14	0.15–0.19	0.20–0.24	0.25–0.29	0.30–0.34	0.35–0.39	0.40–0.44	
S-34-37-3-1	*					1	8	1					.150
S-34-37-4-1	*						4	6					.180
S-34-38-1-1	.05					6	4						.120
S-34-38-1-2	.04					7	3						.115
S-34-38-1-3	.08						10						.150
S-34-38-1-4	.05						10						.150
S-34-39-1-1	.00		7		1	4	18	6		3			.135
S-34-39-1-2	.00		6		3	3	16	4					.116
S-34-39-1-3	.00		77										.000
S-34-39-1-4	.00		78										.000
S-34-40-1		1.20					4	17	1			2	.177
S-34-41-1		1.25				4	17	6	3				.144
S-34-42-1-1	.04						3	6	1				.190

* Parent plant eaten by rabbits.

The striking feature of these results was, as is shown in Table 3, that in progenies of two selections all the plants gave a negative test. These two selections were numbered S-34-39-1-3 and S-34-39-1-4, and in the previous year the parent plants had tested 0.00%. It therefore appeared that these 2 lines were pure breeding for at least a low coumarin content. It was, however, rather surprising that the 2 other plants selected from S-34-39-1, and which also gave negative tests the previous year, produced only a few coumarin-free plants. The behaviour of these 2 plants was very similar to that of their parent strain in that 7 and 6 plants out of 39 and 32 plants, respectively, were free of coumarin, while the remainder gave normal tests. It is also noteworthy that the remainder of the lines, parents of some of which were selected for low, and some for high coumarin, exhibited no practical differences in coumarin content. The progenies of the 2 plants which rabbits had eaten very readily were found to contain normal amounts of coumarin.

In the fall of 1937, there were taken into the greenhouse 25 plants of each of the 2 strains which appeared to be pure breeding for lack of coumarin. Other material taken in included plants from the high testing lines. In tests made during the winter at the flowering stage all plants of strain S-34-39-1-3 gave a negative test except 2 which tested 0.02 and 0.04% coumarin. In line S-34-39-1-4, coumarin percentages varying between 0.01 and 0.075 were found in 16 of the 25 plants. The latter strain was therefore considered to be slightly more variable than the former.

Because of its apparent greater uniformity, strain S-34-39-1-3 was used in establishing an increase plot of low-coumarin sweet clover. Progeny of 14 of the most desirable plants were set out in the spring of 1938 in a plot which contained 1138 plants. In addition, sister plants of these lines were set out in the nursery, together with progenies from plants of strain S-34-39-1-4 and other low and high coumarin selections.

In the fall of the same year coumarin tests were made on the foliage of this material. The results of this testing can be summarized as follows: lines from strain S-34-39-1-3 averaged 0.040% in the nursery and 0.019% in the increase plot, while the average for strain S-34-39-1-4 in the nursery was 0.009% coumarin. Although it appeared that the latter line was lower in coumarin content than the line selected for increase, it is doubtful if the difference is a real one.

In 1939 every plant in the increase plot was again tested for coumarin content. The tests were made in the early flowering stage, the data being summarized in Table 4.

TABLE 4.—THE FREQUENCIES OF SWEET CLOVER PLANTS OF THE LOW-COUMARIN INCREASE PLOT IN CLASSES OF COUMARIN PERCENTAGE. THE PLANTS WERE TESTED IN 1939 IN THE EARLY FLOWERING STAGE

Strain No.	Coumarin percentage											
	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.10	0.15	0.20	Ave.
S-34-39-1-3-1		4	13	9	7		2	1	1			.036
S-34-39-1-3-2		1	1	12	18	2	1	3	1			.041
S-34-39-1-3-3		11	20	20	14	6		4	1	1		.034
S-34-39-1-3-4	58	19	11	5	5	1	1	3				.011
S-34-39-1-3-5	5	34	40	18	10	6	4	6	1			.026
S-34-39-1-3-6	18	3	3	4	7	6			1	1		.024
S-34-39-1-3-7	3	25	20	5	8	4		2				.022
S-34-39-1-3-8	22	18	8	7	8	1		1	1			.017
S-34-39-1-3-9		51	19	3	9	3		5	3		2	.027
S-34-39-1-3-10		13	7	9	3				1			.023
S-34-39-1-3-11	12	31	13	5	2	4	1	7	1	2		.026
S-34-39-1-3-12	2	16	23	10	5	2	2	1				.023
S-34-39-1-3-13		13	11	4	3	1			1			.022
S-34-39-1-3-14		23	10	2	2	2	1	3				.022
Total	120	262	199	113	101	38	12	36	12	4	2	.0253

The average coumarin content of the plot was 0.0253% which is approximately $\frac{1}{10}$ of the amount found in unselected material. As shown by the table there were differences in the coumarin content of different progenies. While these may indicate possibilities for further improvement, it is undoubtedly true that some fluctuations were due to variations in technique rather than to heritable differences.

It has been noted that the original selection (1933–34) and the selections from the next generation (1935–36) were made upon the coumarin content of the seed, and that there were marked differences in the coumarin content of the seed of progenies of high and low coumarin parents. From the fall of 1937 onward the procedure of testing the seed was modified to provide for an incubation period, as suggested by Roberts and Link (9). After adopting the modified test it was surprising to find that consistent significant differences between the high and low coumarin lines did not appear. Some strains which gave very low tests on the foliage gave tests on the seed which were just as high as that of strains having a high coumarin content in the foliage. It appeared that the inconsistency between the results of early work on the seed and that of the later results was due to the change in technique of testing. Confirmation of this was obtained by testing seed of *M. dentata*, unselected Arctic, and a low coumarin line (Pioneer), by the original method which involved no incubating, and by the modified method which included an incubation period. The average results from 7 samples of each variety are given in Table 5.

TABLE 5.—COUMARIN CONTENT OF SEED WHEN EXTRACTED BY TWO DIFFERENT PROCEDURES

Extraction procedure	Average percentage coumarin in seed of		
	M. dentata	Low coumarin strain	Arctic
Not incubated	0.00	0.03*	1.16
Incubated	0.22	0.59	1.22

* In 4 of the 7 samples the test showed 0.00%.

As shown by Table 5, in the case of *M. dentata* and the low-coumarin strain, hydrolysis of the seed sample before extraction gave a very marked increase in the test for coumarin. This appears to support the contention of Roberts and Link that in the seed a proportion of the coumarin is present in the bound form which is only released by enzymatic action. However, if we accept this explanation of the results from samples of *M. dentata* and the low coumarin strain, we must conclude that there is practically no bound coumarin in seed of the Arctic variety. Such a conclusion appears to be biologically unsound since the low coumarin strain is a deviation of the Arctic variety, and it seems unreasonable to expect that they would differ so greatly in the proportion of the total coumarin which is in a bound form. As an alternative to the above explanation, the results may be interpreted as indicating that through incubation, compounds other than coumarin are released, which react in the same way in the test as does coumarin. However, the failure to obtain a materially higher test in the case of incubated seed of the Arctic variety is evidence against the acceptance of this interpretation. It is apparent that while no definite conclusion can be drawn from the data presented in Table 5, it serves to emphasize the need for a carefully conducted investigation of bound coumarin in the seed. The results do show that the discrepancies between the early tests on seed and those tests in later years arose largely through the change in extraction procedure.

The above data show that a reduction in coumarin content of the seed has been brought about by selection. The improvement is apparently not as great as that effected in the foliage.

INHERITANCE OF THE LOW-COUMARIN CHARACTER

Since the progenies of some low coumarin selections had been found to breed true for the character, it appeared that it was heritable. To secure data on the mode of inheritance, crosses were made between low and high coumarin selections in 1938. The F_1 plants were more or less intermediate in coumarin content. F_2 progenies were set out in the spring of 1939 and a portion of them tested in the fall of their first year's growth. The coumarin percentages of the 209 F_2 plants tested, and the progenies of their parents are shown in Table 6.

The table shows that the F_2 plants fell into two fairly distinct classes, being either high or quite low for coumarin content. There were few intermediates. Assuming a 1-factor hypothesis, the P value for the above data is 0.65. However, the study is not considered extensive enough to be conclusive. Complete results of the inheritance study will be presented in a later paper. The data are included here for the purpose of showing that the low-coumarin character is inherited as a recessive in an apparently simple manner.

TABLE 6.—FREQUENCIES OF COUMARIN PERCENTAGES IN PARENT CHECKS AND IN F_2 OF CROSSES BETWEEN HIGH AND LOW COUMARIN SELECTIONS AND THEIR PARENTS

Strain No.	No. of plants with coumarin percentage of											
	0.00	0.01	0.02	0.03	0.04	0.05	0.10	0.20	0.30	0.40	0.50	0.60
Selfed Progenies of Parents												
S-34-39-1-3-8	11	25	2	1		2						
S-34-40-1-3					1			1	4	10	12	1
F_2 Progenies												
C-38-I-5-1*	16	2	7	2	1	3	1	7	5	14	14	27
C-38-I-21-1**	1	5	5	6	6	1		12	16	29	21	8
Total of F_2	17	7	12	8	7	4	1	19	21	43	35	35

Total No. F_2 plants with between 0.00 and 0.05% coumarin = 55

Total No. F_2 plants with between 0.20 and 0.60% coumarin = 153

*F_2 of S-34-39-1-3-8 × S-34-40-1-3.
**F_2 of S-34-39-1-3-9 × S-34-40-1-3.

THE CROSSING OF LOW-COUMARIN SPECIES WITH *M. alba*

Brink (1) in 1934 reported that *M. dentata* was coumarin-free and he consequently called the species non-bitter sweet clover. A large collection of introductions of this species have been grown and tested for coumarin at Saskatoon. Many were found to be practically free of coumarin, the tests of the low coumarin lines and this species being often very similar. However, none of the *M. dentata* introductions have had agronomic characteristics which would enable them to displace common sweet clover as a cultivated crop. Attempts were therefore made to transfer the low coumarin character of *M. dentata* to *M. alba* by crossing. Stevenson and Clayton (12) reported failure to obtain successful crosses in the early part of

this work. Since then crosses have been extensively tried between *M. dentata* and *M. alba*, *M. suaveolens* and *M. officinalis*. In each case pollinations were made in both directions using practically all available varieties of the species. Crossed seeds were obtained fairly frequently, irrespective of the direction of the cross. However, if these seeds were viable they invariably gave rise to weak and albino seedlings which died within 2 or 3 weeks after emergence.

DISCUSSION

Through continuous selection in inbred lines a strain of sweet clover has been developed, the foliage of which contains only about $\frac{1}{10}$ the amount of coumarin found in ordinary sweet clover. Although in this line there has evidently been a marked decrease in coumarin content, it is not completely free of the substance. For instance, in 1939 out of 861 plants tested in the low coumarin increase plot only 21 contained 0.10% or more coumarin. The coumarin content of the seed also shows a substantial reduction from that found in ordinary sweet clover. Tests on the buds and flowers have shown that these parts may contain as high as 0.6 and 0.4% coumarin respectively. Experience in 1938 indicated that there may be a tendency towards the formation of coumarin under adverse circumstances. Thirteen of the low coumarin plants brought into the greenhouse in the fall of 1937 were kept there until late June, 1938. Watering was discontinued towards

FIGURE 1. Single plant in increase plot on June 23, 1939—about 4 feet high.

FIGURE 2. Single plant in increase plot on Aug. 10, 1939. Screen is 5 feet 8 inches high.

the end of May. Tests of the foliage on June 13 showed an average coumarin content in these plants of 0.15%. A somewhat similar tendency was noted in a few plants remaining in the field in 1938. Tests of these plants in June and July were very low but some of them appeared to contain substantial amounts of coumarin in late August. However, variations in the test used are known to occur. It may well be, therefore, that some of these apparent variations in the coumarin content of the plants are actually due to variation in the test.

The selection for high coumarin content appears to have failed to increase the amount of coumarin over that found in the foliage of unselected material. However, this selection has been practised in only 4 lines which were originally selected on the basis of high coumarin seed.

The low coumarin line is a derivative of the Arctic variety and in its morphological characteristics is quite similar to it. Although selfing in sweet clover sometimes results in a loss of vigour, the low coumarin strain appears to be quite as vigorous as its parent variety. This fact and the plant type can be noted from Figures 1 and 2 which show single plants of the strain in the increase plot. It should be noted that these plants were grown in spacing 3 feet by 3 feet under favourable moisture conditions.

The low coumarin strain has been licensed in Canada as the variety "Pioneer", and will be increased under contract in 1940–41.

SUMMARY

1. Beginning in 1933–34, selection has been carried on continuously towards the production of low coumarin lines of *M. alba*. Clayton and Larmour's colorimetric method has been used throughout in testing for coumarin.

2. In 1936, after one year's selfing several plants appeared which in their first year of growth tested 0.00% coumarin. Some of these plants have proved to be pure breeding and have been increased.

3. The increased line appears to have a coumarin content of between 0.00 and 0.05% and to average around 0.02%, which is about $\frac{1}{10}$ of that in unselected material.

4. No increase in coumarin content has been secured through selection.

5. Crosses between low and high coumarin lines show that the low coumarin character is definitely inherited and is probably dependent upon a single recessive factor.

6. Attempts to transfer the low coumarin character of *M. dentata* to other sweet clover species and varieties have been unsuccessful due to the failure to get crosses.

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INVESTIGATIONS CONCERNING THE COUMARIN CONTENT OF SWEET CLOVER[1]

II. SOURCES OF VARIATION IN TESTS FOR COUMARIN CONTENT

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Stevenson and White (5) have traced the history of the development of a low coumarin strain of sweet clover *(M. alba)*. To a critical reader several notable irregularities in the coumarin tests reported therein will appear. Peculiarities were noted in the various coumarin analyses of the vegetative material. For instance, in 1936 four plants were selected, two of which contained .05 and two .04% coumarin, but in 1937 their selfed progeny contained normal amounts of the substance. Also, although low coumarin appears to be a recessive character, two plants which gave negative tests in 1936 produced selfed progenies in 1937 which were segregating for the low coumarin character.

The purpose of this paper is not to attempt to explain away these irregularities but to present some additional information on variation in coumarin content of sweet clover plants, and to examine some of the data from the standpoint of accuracy of the test.

Variations in the coumarin content found in sweet clover plants could be caused in any one or a combination of the following ways:—

(1) Variations in the coumarin content of different parts of the sweet clover plant.

(2) Variations in the coumarin content of sweet clover plants in different stages.

(3) Inaccuracies or variations caused by the test used to determine coumarin.

Throughout the investigations reported in this paper the technique of sampling, extracting, and testing was the same as given by Stevenson and White (5), unless otherwise stated.

VARIATIONS IN THE COUMARIN CONTENT OF DIFFERENT PARTS OF THE SWEET CLOVER PLANT

All of the methods now used for determining coumarin in sweet clover plants involve the use of a sample, usually under 5 grams. One of the most recent methods proposed, that of Roberts and Link (3), involves the use of only a portion of 4 leaflets. It is obvious that in securing samples of such dimensions from a plant which may weigh several pounds, variations in content of the different parts of the plant would be of extreme importance. In 1936 Stevenson and Clayton (4) showed that the leaf material of sweet clover plants contained approximately three times the amount of coumarin that was found in the stem. Since that time sampling for coumarin determinations has been confined to the leaves. In 1939 some variations

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occurred which were suspected of being due to sampling. Accordingly 10 plants were selected for study and coumarin determinations made on each of the following material from each plant: (1) old leaves from near the base of branches, (2) newly formed leaves from near the apex of branches, (3) buds, and (4) blossoms.

Table 1.—The percentage of coumarin in different parts of sweet clover plants

Plant No.	Description of plant	Percentage coumarin in:							
		Old leaves		New leaves		Buds		Flowers	
		Sample No.		Sample No.		Sample No.		Sample No.	
		1	2	1	2	1	2	1	2
1	*M. alba*	.15	.15	.4	.4	.6	.6	.3	.3
2	*M. officinalis*	.04	.04	.4	.5	.6	.6	.3	.3
3	High coumarin selection	.1	.1	.4	.2	.6	.7	.5	.5
4	Low coumarin selection	.02	.02	.02	.02	.6	.6	.4	.4
5	Low coumarin selection	.02	.02	.02	.02	.6	.6	.15	.4
6	Low coumarin selection	.01	.01	.01	.01	.4	.4	.2	.2
7	Low coumarin selection	.01	.01	.04	.05	.6	.6	.2	.2
8	Low coumarin selection	.02	.02	.02	.04	.4	.4	.15	.15
9	Low coumarin selection	.03	.02	.02	.075	.5	.4	.2	.2
10	Low coumarin selection	.02	.02	.15	.1	.4	.4	.2	.2
Average		.04		.15		.53		.27	

Determinations were made in duplicate with the results shown in Table 1. There is a strong indication that newly formed leaves have a higher coumarin content than the older leaves near the base of the stem. This was especially marked in plants of normal coumarin content, and not so noticeable in plants of low coumarin selection. The coumarin content of the bud was remarkably high, varying between .4 and .7% of the green weight. Strangely enough this was considerably reduced in the flower, varying between .15 and .5%. These results would indicate that sampling is important. The inclusion of a small part of the bud or flower, or the selection of the upper leaves of a branch in one instance and the lower ones in another would materially affect the results.

It might be added that tests made on similar leaves of different branches showed that no important differences existed between different branches of a plant.

VARIATION IN THE COUMARIN CONTENT OF SWEET CLOVER PLANTS IN DIFFERENT STAGES

Ufer (7) in 1934 recognized a variability in coumarin content of sweet clover plants at different times. He gave the following summarization of these variations:—

Early growth stage, first year...........low coumarin
Fall of first year.......................high coumarin
Early stages of second year.............low coumarin
Mature stages of second year...........high coumarin

Both Suvorov (6) and Gelcinskaja and Bordunova (2) considered that the maximum coumarin content was attained at flowering. Stevenson and Clayton (4) in 1936 reported results of investigations in this connection. The sweet clover plants which they studied were divided into three types. In all three types, in the second year of growth, the highest coumarin content was in the early stages with the lowest at maturity. With the exception of the early growth stages, the coumarin content was as high at late budding or early blossoming stages as at any other time. Variations from the early growth to late budding stage, and from then to maturity, were the main differences between the three types. They also presented data which indicated that in the first year of growth the highest coumarin content occurred in the 4-leaf stage, and was somewhat lower in the fall, but the difference was not consistent.

In the summer of 1936 tests were made on 31 varieties and strains of sweet clover at different growth stages during their second year. Different plants were sampled to obtain the coumarin content at different stages. It would therefore be possible for a difference in coumarin content of plants to affect the comparisons between the different stages. However, where the average of a number of plants is taken such differences are not likely to have much effect. The data secured are shown in Table 2. In this case the coumarin content was definitely highest in the very early growth stages and lowest at maturity. There was a slight decrease after the early stage followed by another peak at budding, and from there on there was a steady decrease to seed setting.

TABLE 2.—A SUMMARY OF TESTS SHOWING THE AVERAGE PERCENTAGE COUMARIN IN SWEET CLOVER PLANTS AT DIFFERENT STAGES OF GROWTH

Date of testing	Growth stage	Number of plants tested	Average percentage coumarin
May 13–14	Very early, $1\frac{1}{2}$–4″ high	13	.513
May 20–21	4 weeks from budding	24	.343
May 28–29	3 weeks from budding	26	.417
June 2– 3	2 weeks from budding	21	.285
June 9–10	1 week from budding	31	.256
June 15–18	Budding stage	31	.345
June 22–23	1 week to flower	31	.291
July 2	10–50% flowering	31	.195
July 14	Early seed setting	31	.125
July 21–23	Seed setting	31	.111

In 1939 one hundred plants in the low-coumarin increase plot were marked for weekly testing. Because of the small amounts of material required and the vigorous growth of the plants, possibilities of the repeated samplings affecting them were negligible. At no time could the effect of sampling be observed on the plants. The data obtained are presented in Table 3 in a frequency table. In this case the maximum amount of coumarin appeared at the budding stage, and again it fell off to a much smaller amount as seed setting set in. The agreement between these two

tables and the observations of Stevenson and Clayton (4) would all indicate that the budding or early flowering stage is a satisfactory time for testing since there is a tendency for coumarin content to be at about the maximum at that time.

It is apparent that if considerable variation existed in the stage of development of the material being tested, this could cause fluctuations in the results. In order to avoid this source of error the plants should be tested at as nearly the same stage of development as is possible.

TABLE 3.—THE PERCENTAGE OF COUMARIN IN 100 PLANTS TESTED AT WEEKLY INTERVALS

Date tested 1939	Height of plants	Stage	Percentage coumarin											
			.00	.01	.02	.03	.04	.05	.06	.08	.10	.15	.20	Ave.
May 15	14″		80	12	2	5	1							.004
May 22	16″		64	29	6		1							.004
May 30	24″		54	27	14	4	1							.007
June 12	—		22	22	13	5	12	8	3	8	7			.030
June 19	43″	Bud			12	24	34	5		17	6	2		.048
June 26	—	10% flower	22	32	11	14	4	7		5	2	3		.025
July 3	59″	—	27	36	15	6	3	3	1	2	5	2		.021
July 10	—	Flower	3	39	21	5	4	4		8	9	4	3	.041
July 17	—	Full flower	12	39	39	7	1	1				1		.016
July 24	73″	Early seed setting		11	54	25	6	2		1		1		.025
July 31	—	Early seed setting	1	29	40	17	8	4		1				.022
Aug. 7	—	Seed setting	1	58	27	4	6	4						.017

INACCURACIES OR VARIATIONS APPARENTLY DUE TO THE TEST ITSELF

Both Clayton and Larmour (1), and Stevenson and Clayton (4) state that the method first outlined by the former authors for coumarin determination is not strictly quantitative. The reason is that the presence of plant pigments and possibly also of phenolic compounds makes accurate colour comparisons difficult. That inaccuracies do occur was shown definitely in the 1938 and 1939 tests on the low-coumarin increase plot.

In 1938 it was noted that there was a tendency for the readings to run in groups of 10; a series of 10 consecutive plants might give a rather high test, while a following 10 might give a very low test. In testing this material it had been the practice to boil, shake, etc., the samples in groups of 10. By referring to the original records it was found that the grouping of the readings coincided with the groups in which the tests were made. In 1939 the procedure was slightly altered, and 20 plants were put through the test in a group. Again it was noted that the readings fell into groups coincident with the testing of the material. In Table 4 the distribution of the groups tested according to their average coumarin percentage is shown.

Analysis of variance of the data was made with groups as the only criterion. The following results were obtained:—

1938 F value for groups = 13.12 5% point = 1.37
1939 F value for groups = 127.14 5% point = 1.41

It is recognized that such analysis of this data is not strictly accurate, particularly since groups of plants were taken along a row, and are therefore confounded with strains. However, the above F values were calculated after having subtracted the sum of squares for strains from that for groups.

TABLE 4.—THE DISTRIBUTION OF GROUPS IN WHICH SWEET CLOVER PLANTS WERE TESTED IN 1938 AND 1939 ACCORDING TO THEIR AVERAGE COUMARIN CONTENT

Class of coumarin percentage	1938 Number of groups of 10 plants	1939 Number of groups of 20 plants
.000 – .003	21	3
.004 – .007	8	2
.008 – .011	14	4
.012 – .015	13	3
.016 – .019	9	7
.020 – .023	8	6
.024 – .027	6	5
.028 – .031	3	3
.032 – .041	2	5
.042 – .051	3	2
.052 – .061	0	1
.062 – .071	2	

Therefore the above F values represent the minimum degree of significance for differences between group means because there would probably be some group differences taken out in the sum of squares for strains. In connection with these analyses it is also interesting to note that the S.E. of a single determination, including group differences in error, was .021 in 1938, and .020 in 1939. Therefore it would be expected that about 5% of the plants would show coumarin content of over .04% due to unknown variations in the test. Also a difference of roughly .06% coumarin between two plants would be necessary for significance.

During both years detailed notes were made for each group as to the time of sampling, time tested, time read and so on, but there was no apparent tendency for variations to be related to any of these factors.

Following the analysis of the 1938 results, technique was studied by varying alcohol concentrations, length of time and intensity of boiling, length of time between sampling and grinding, and length of time between grinding and extraction. Only the first of these, alcohol concentration, seemed to have much effect upon the test. During the 1939 testing on three occasions a group was allowed to stand over night in the 50% alcohol solution before boiling, and the averages of the three groups were: .060%, .042%, .092%. This feature was retested but did not give consistent results. However, it appears that this practice may result in higher reading and be a source of variation. From the study of technique it may be said that no definite clue was discovered as to cause of the variations noted as arising out of the test itself.

From the data on 528 plants in the low-coumarin increase plot a determination was made of the simple correlation coefficient between the coumarin content in the fall of the first year's growth (1938), and the coumarin content at the early flowering stage of the second year's growth (1939). The surface is shown in Table 5, r being found to equal $-.20$, a non-significant value. This is well illustrated in the table where there seems to be no relation between the two years' tests. This complete lack of a correlation would indicate that unless the plants vary haphazardly from year to year in coumarin content the test, as it was used, is not accurate to within at least .10%.

TABLE 5.—THE CORRELATION IN COUMARIN PERCENTAGES OF PLANTS IN THE LOW-COUMARIN INCREASE PLOTS, SHOWN BY 1938 AND 1939 TESTS

Percentage coumarin 1938 test	Percentage coumarin in 1939 test												Number of plants
	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.10	0.15	0.20	0.25	
.00	51	57	50	55	42	20	5	13	8	2			303
.01	8	24	27	7	12	3	2	4	2				89
.02	9	13	18	12	5	4	1	1	1				64
.03	1	2	6		2		1		1				13
.04	6	3	4	1	1	1							16
.05	4		2	3	2	2		1			1		15
.06	4	2			1								7
.08	1	2		2	2								7
.10			2	3	1			1					7
.15	2				3								5
.20													
.25		1			1								2
Number of plants	86	104	109	83	72	30	9	20	12	2	1		528

TABLE 6.—THE CORRELATION IN COUMARIN PERCENTAGES OF SWEET CLOVER PLANTS FROM THE COUMARIN NURSERY, AS SHOWN BY 1938 AND 1939 TESTS

Percentage coumarin 1938 test	Percentage coumarin in 1939 test															Totals
	0.00	0.01	0.02	0.03	0.04	0.05	0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80	0.90	
.00	15	29	22	7	5	6	5	1								90
.01	3	6	2	2	1	1										15
.02	9	10	2	1	2	1	2	1								28
.03	4	11	7	8	3	2	2									37
.04	4	7	3	2	3	3	1									23
.05	3	9	3	3	4											22
.10	1	2	1				1									5
.20								5		6						11
.30								7	3	3	1					14
.40								2	4	3		1			2	12
.50								1	4	1	1	1		1		9
.60								3	2	6	1	3			1	16
.70									2	1						3
.80																
.90																
Totals	39	74	40	23	18	13	11	20	15	20	3	5		1	3	285

On the other hand, a correlation between the 1938 and 1939 tests on plants in the coumarin nursery showed an r of $+.83$, a highly significant value. The surface for this correlation is shown in Table 6, showing the 1938 and 1939 coumarin tests on 285 plants. In this case, however, there were included not only plants of low coumarin content but also plants of high coumarin lines. In both years these were clearly separated, and thereby gave rise to the highly significant correlation coefficient. In low-coumarin material it seems likely that variations arising out of the test itself will camouflage any heritable differences that may exist. It can be definitely said, however, that the test will readily distinguish between low and high coumarin plants or strains.

Variations in the seed tests have also been quite marked. Stevenson and White (5) have shown that the discrepancy between the results in the early seed testing and that of later work arose through adoption of a modification of the extraction procedure. Until the fall of 1937 no incubation was given in extracting coumarin from the seed, but since 1937 Roberts and Link's (3) recommendation to incubate for one hour at 40° C. has been followed. The incubation appeared to have little effect on the results from seeds containing normal or high amounts of coumarin, but seeds from low-coumarin material gave much higher readings when incubated.

In conclusion, it may be said that the test outlined by Clayton and Larmour, and used extensively at Saskatoon, has been invaluable, in that it enabled the selection of a low-coumarin sweet clover. However, for further improvement and the development of a coumarin-free strain it would appear that a test which could more accurately reflect small amounts of coumarin would be desirable.

SUMMARY

1. Coumarin content was found to vary in different parts of the sweet clover plant. Newly formed leaves showed a higher content than old leaves, and the buds were found to have an extremely high content. These results demonstrate that sampling may be an important source of variation.

2. Tests on plants in different stages of growth showed that the coumarin content followed a certain trend as growth progressed. At the late budding or early flowering stage the content was found to be at about the maximum, and thus this is a very satisfactory stage at which to test. Variations in the stage of testing may cause discrepancies in the results.

3. Considerable variation was traced to the test itself. The source of these variations arising from the test could not be ascertained.

4. For breeding towards further reduction or elimination of coumarin a more refined test would be desirable.

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TURKESTAN ALFALFA AS A MEDIUM OF WEED INTRODUCTION[1]

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Turkestan alfalfa seed has long been known to be a carrier of the seed of Russian knapweed, *Centaurea repens* L. (*C. Picris* of many earlier publications), to North American fields sown to it. It has been imported at times to the extent of several millions of pounds annually, according to Oakley and Westover (17) who also say "commercial Turkestan alfalfa seed can be identified in most cases by the seed of Russian knapweed which it almost invariably contains."

While Russian knapweed as an indicator species has received most publicity, it has not been the only weed coming with Turkestan alfalfa. Wilcox and Stevenson (24) in 1909 and Musil (16) quite recently have listed weeds associated with Russian knapweed in alfalfa seed. Among the weeds are some of familiar occurrence already in America, others still unknown except from occurrence in seed samples examined, and still others, like the knapweed, definitely additions to our weed flora.

In another paper (10) the discovery of two hoary cresses new to Canada, *Cardaria Draba* (L.) Desv. var. *repens* (Schrenk) O. E. Schulz (lens-podded hoary cress) and *Hymenophysa pubescens*, C. A. Mey (globe-podded hoary cress) has been reported; and because of the number of occurrences of one or both in association with Russian knapweed the assumption was made that Turkestan alfalfa was the medium of introduction. In this paper the evidence for the position taken, and some of the relevant history of a rather distinct influx of weeds is presented.

HISTORY

In 1898 Prof. N. E. Hansen, as a special agent of the United States Department of Agriculture, secured from various parts of the extensive territory of Russian Turkestan, the seed which during the next year or two was distributed through most of the States and Territories of the Union. Kennedy (13) points out that "owing to the primitive conditions prevailing in the region in which the seed was collected, it was not clean when purchased, and had to be carefully inspected and freed from all weed seeds before being distributed." There is nothing at hand to show that weeds became established through this distribution of seed, but in a very few years seed from one part or another of Turkestan was being handled commercially and without equal care to insure freedom from weed seeds. In a discussion of certain phases of this trade Brown (2) states that "Over 95 per cent of the alfalfa seed received since July 1, 1913, came from

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Turkestan," and "it seems a conservative estimate to assume that one-tenth of the 5,000,000 or more acres of alfalfa now growing in this country was planted with commercial Turkestan seed." It was also considered safe to say that "If any seeds of Russian knapweed occur, the alfalfa seed is wholly or in part from Turkestan."

The remarkable preponderance of Turkestan among imported alfalfa seeds by 1914 has not been maintained uniformly, if at all, and the peak of importation probably has not been reached in all parts of the continent at the same time. The early trials soon demonstrated its unsuitability for eastern more humid regions. Incidentally, it was sown in Canada in 1900 at the Dominion Experimental Farm, Indian Head, Sask., (14), and while wintering well, and used at other of the experimental stations at later dates, it has been superseded by more satisfactory home-bred strains. According to Johnson (12) "the outbreak of war in 1914 stopped the source of supply", and while in California that seemed to mark the end of the trade, he remarks that "other localities are still (1923) receiving seed from this source". He quotes Hensel and Harling (11): "The sale of Turkestan seed has increased greatly in Kansas in the last year (1921–1922) and samples sent to the Seed Laboratory by farmers show that some of it contains knapweed seed". While no longer occupying the place it once did in America, it appears from studies of Peltier in Nebraska (18) that Turkestan alfalfa had still in 1933 kept a special niche for itself as one of the few strains of "wilt" resistant alfalfas.

While Turkestan alfalfa seed was probably coming into both eastern and western Canada for some years previously, it was not until 1910, in samples from Petrolia, Ont., and in 1914, from Lindsay, Ont., that Russian knapweed seeds were detected at the Division of Botany. About 1920, according to Miss A. W. Anderson, then on the staff of the Dominion Seed Laboratory, Winnipeg, Man., some of the characteristic white seeds were appearing in Turkestan alfalfa seed there. Dr. F. T. Wahlen, at that time Chief Seed Analyst, expressed the opinion in conversation some years ago that further advent of weeds through this channel was unlikely after about 1922; and evidence of any recent contamination with Russian knapweed is lacking. The only known resulting infestation in eastern Canada is one at Newmarket, Ont., found in 1933. From western Canada specimens began coming to the Division of Botany in 1928 from both Alberta and Saskatchewan, and soon from the other provinces—all years after their probable advent.

Some personal experience on an Ontario farm with the common alfalfa seed secured before 1907, and with other seed obtainable for a few years following, proved the latter to give distinctly less satisfactory short-lived stands. These later seedings, moreover, came up infested with a mustard which was identified later by Dr. James Fletcher, Central Experimental Farm, Ottawa, as garden rocket, *Eruca sativa* Mill., an importation with alfalfa seed from southern Russia as then reported. Then, and for a few years following, the tell-tale weed was being noticed in both eastern and western Canada (15, 8), but apparently was not becoming naturalized, and after a few years was no longer seen except as traced occasionally to culture in gardens for salad purposes. In one instance, at Indian Head, Sask., it was reported from a station from which later Russian knapweed was

also reported. In view of other apparently significant weed associations with knapweed to be discussed, the possibility is suggested of a like source for another weed, perennial rape, *Rapistrum perenne* (L.) All., which has spread through a number of townships around Grenfell, Sask., another of the western stations for *E. sativa* (9). This weed has, like each of the above, a Russian distribution, and seems not to have been reported elsewhere in in North America.

The association, already mentioned, of the hoary cresses with Russian knapweed came to notice after curiosity had been aroused over the repeated occurrence together of the hoary cresses. Examination of records for a period beginning about 1928 which was notable for the number of new weeds brought to the attention of the Division, revealed an instance in which *C. Draba* var. *repens* and Russian knapweed were sent by the same farmer, and another in which *H. pubescens* and the knapweed were sent from an adjoining province by another farmer. Similar associations were soon found involving all three, as at Cawston, B.C., where they are reported all growing in a field and adjacent orchard.

Another weed with widespread (including western Asian) distribution, and known in Canada from the British Columbian interior, is red orache, *Atriplex rosea* L., which also tends to occur in districts having Russian knapweed. According to Eastham (4) it is abundant in the lower Okanagan and has been found north to Enderby, and specimens were collected personally about 12 miles east of Kamloops in 1939. It figures as a weed in States to the south of the province, and very locally in eastern States.

Still another weed, a species of the Caspian Sea region, *Bassia hyssopifolia* (Pall.) Kuntze, has appeared during the period in question in some proximity here to several of the associated weeds. In view of its discovery at Kamloops in 1920 (3) the year after it was first detected in America in Nevada (1), and its being reported within a year or two at places from Washington to California, some widely spread carrier such as Turkestan alfalfa has been shown to be, offers a better explanation of its introduction than would a theory of spread from one or two primary sources. The same reasoning could be applied to others of the newer weeds, but at least a direction has been indicated for further investigation. In Table 1 joint occurrences of the various species at a station are shown in no less than 15 instances, as many as 4 occurring at Kamloops, B.C. Except where recorded by "R", specimens are in Canadian herbaria and have been seen.

Table 1.—Distribution in Canada of species under discussion

Weeds (All with southern Russian distribution)	*Cardaria Draba* Heart-podded Hoary Cress	*Cardaria Draba* var. *repens* Lens-podded Hoary Cress	*Hymenophysa pubescens* Globe-podded Hoary Cress	*Centaurea repens* Russian Knapweed	*Eruca sativa* Garden Rocket	*Rapistrum perenne* Perennial Rape	*Bassia hyssopifolia* Thorn Orache	*Atriplex rosea* Red Orache
Yarmouth, N.S.	x							
Cap a L'Aigle, Que.	x							
Ottawa, Ont.	x				x			
Chesterworth, Ont.	x							
Lindsay, Ont.				x				
Newmarket, Ont.				x				

TABLE 1.—DISTRIBUTION IN CANADA OF SPECIES UNDER DISCUSSION—*Concluded*

Weeds (All with southern Russian distribution)	*Cardaria Draba* Heart-podded Hoary Cress	*Cardaria Draba* var. *repens* Lens-podded Hoary Cress	*Hymenophysa pubescens* Globe-podded Hoary Cress	*Centaurea repens* Russian Knapweed	*Eruca sativa* Garden Rocket	*Rapistrum perenne* Perennial Rape	*Bassia hyssopifolia* Thorn Orache	*Atriplex rosea* Red Orache
Barrie, Ont.	x							
Markdale, Ont.	x							
Walkerton, Ont.	x							
Guelph, Ont.	x							
Preston, Ont.					x			
Galt, Ont.	x				x			
Niagara Falls, Ont.	x							
London, Ont.					x			
Ewart, Man.				x				
Brandon, Man.	x							
Weyburn, Sask.				x				
Maxim, Sask.				x				
Broadview, Sask.						x		
Grenfell, Sask.					x	x		
Indian Head, Sask.	x			x	x			
Regina, Sask.					x			
Semans, Sask.					x			
Kelliher, Sask.			x	x				
Sutherland, Sask.			x	R				
Saskatoon, Sask.		x						
Traynor, Sask.		x						
Landis, Sask.		x		x				
Scott, Sask.			x					
Calderbank, Sask.				x				
Swift Current, Sask.			x					
Gull Lake, Sask.				x				
Medicine Hat, Alta.				x				
Carseland, Alta.		x	x	x				
Chestermere Lake, Alta.			x					
E. of Calgary, Alta.		x						
Chauvin, Alta.				x				
Galahad, Alta.				x				
Fort Saskatchewan, Alta.				x				
Grimshaw, Alta.			x					
Grande Prairie, Alta.		x	x					
Rolla, B.C.			x					
Pouce Coupe, B.C.			x					
Trail, B.C.	x							
Cawston, B.C.		x	x	x				
Penticton, B.C.				R			x	x
Summerland, B.C.		x						x
Vernon, B.C.	x	x		R				
Armstrong, B.C.	x							
Enderby, B.C.								x
Monte Creek, B.C.							x	
Kamloops, B.C.			x	R			x	x
Tranquille, B.C.				x			x	
Salmon Lake, Nicola Dist., B.C.			x					
Lillooet, B.C.				x				
James Bay Flats, Victoria, B.C.	x							

R. Reported, specimens not seen.

DISCUSSION

It is no doubt significant that the weeds concerned have all an Asian, whether or not a strictly Turkestan, distribution. With the exception of *Rapistrum* and *Bassia*, their seeds are all mong those recorded as being associated with Russian knapweed in alfalfa seed samples. In the case of a third (heart-podded) hoary cress, *C. Draba* (L.) Desv., this would not be the earliest introduction since one collection in 1878 from Barrie, Ont., was labelled as escaped from gardens, and it is reported that a collection in the Gray Herbarium came from California in 1876. In the case of hoary cress, again, the probability has to be entertained that *C. Draba* var *repens* as well as the species, and *Hymenophysa*, may all have passed as one thing. The seeds, being very similar, were not being distinguished up to the time a few years ago, when importations of Turkestan alfalfa ceased. The earliest records for *H. pubescens* include one from Ypsilanti, Mich. in 1919 (23) and one from Idaho in 1925 (21). Until 1936 when it was collected at Philadelphia (6) it had not been found apparently east of the original Michigan station.

It is a fair conclusion, then, that importation of commercial Turkestan alfalfa seed, commencing about 40 years ago, and according to Brown (already quoted) expanding chiefly because of undue retailing profits possible, was responsible largely, when not entirely, for the numerous infestations now existing in both United States and Canada, of Russian knapweed, hoary cress (one or more), rocket (formerly) and doubtless some of these other weeds as well. The considerable lapse of time before infestations began to be reported in numbers need not impair the argument for a Turkestan alfalfa seed origin of these outbreaks, as weeds with perennial root systems are apt to extend rather slowly at first. In alfalfa, cut early and more than once in the season, they show up less strikingly than they would in the cereal crops which may succeed a good alfalfa stand only after some years, and only then perhaps are brought to official notice.

Allowance must, perhaps, be made for spread after introduction from initial points to secondary ones. Good evidence as to the extent of such dispersal is lacking, but its possibility may be illustrated by an experience at the Dominion Experimental Station, Swift Current, Sask. A hoary cress, now identified as *Hymenophysa*, appeared 10 years or more ago outside the Soils Laboratory of that date. It was surmised that it came with soil samples received from other experimental stations and in due course thrown out. Brandon was one of the sources suggested, but the fact being pointed out that the only hoary cress yet found at Brandon was *C. Draba*, the fact was elicited that *Hymenophysa* had been established at the Dominion Experimental Station, Scott, Sask., prior to a date of shipping soil samples from there. Turkestan alfalfa seed had been sown still earlier at both Scott and Swift Current, and only the evidence present of soil sample rather than field origin of the infestation at Swift Current directs suspicion to outside sources. The quest could be pushed back still farther than Scott since records show (5) that not only was Turkestan alfalfa sown in 1911, but the plot was inoculated for alfalfa growing with soil brought from Indian Head. Inasmuch as only *C. Draba* is recorded from Indian Head, it seems unnecessary to look beyond the Turkestan seed sown at Scott for the *Hymenophysa* infestation there, and to all appearances that at Swift Current.

The Turkestan alfalfa carrier of Russian knapweed and associated weeds originated from such diverse conditions in that part of Russia that uniform weed content is by no means to be expected. From many places in America contamination has been reported as very light. In another report by Rogers (19) figures were presented which indicated a more alarming situation. "In March, 1928", he states "there was released from the Denver Custom-house approximately 50,000 pounds of Turkestan alfalfa seed, imported from Russia. About 25,000,000 seeds of Russian knapweed were brought in with this alfalfa seed. This is enough to produce a perfect stand on at least 5,000 acres of land." This would amount to 500 knapweed seeds in a pound of alfalfa seed, and using the estimate of around 225,000 alfalfa seeds to a pound, would mean one knapweed seed to each 450 seeds of alfalfa. No figure for survival probability is at hand for knapweed or for hoary cress, but for rocket, survival after one or two years in Ontario experience was nil or practically so. It was apparently so as well for many of the other species detected in seed testing but not thus far in the field.

In passing, a word should be added in support of the wider use in weed investigations of the considerable data already assembled in seed laboratories and applied to determining the origin of agricultural seeds. The indicator weed seeds while pointing to the source, suitable or otherwise, of the seed to be sown, might also be more widely used in tracing the source of a weed infestation and thus guarding against repetition of such a situation as has been outlined. Musil, whose work has already been cited, and Gentner (7) are among the latest who have contributed in this field. Gentner's treatment, as Musil observes, is "an essentially complete resume of the incidental seeds which have been found to occur in crop seeds from most of the sources of production." Wahlen (22) in Canada has discussed the rôle of weed seed impurities in determining the source of crop seeds, and states that Nobbe in *Handbuch der Samenkunde*, Berlin, 1896, was the first to point out these possibilities. Stebler (20) is credited with inaugurating the use of the principle in seed testing, and along with others, establishing lists of "leading species" for different countries. This work has been more fully done for red clover and alfalfa than for other crops.

By way of reviewing the present situation it may be said that since about 1922 any spread of these weeds, in Canada at least, would be as secondary infestations rather than from further importations, but it is doubtful if any great proportion of the primary infestations have yet been reported. Past invasion was probably centred largely on the periods between 1907 and 1914 and again around 1920. A distribution of alfalfa seed by the Canadian Pacific Railway about 1911–12 is considered by Alberta agricultural officials to have been largely responsible. East of Ontario where alfalfa has been less grown introduction was apparently negligible. East of Saskatchewan no record has been found of the establishment of *C. Draba* var. *repens* or *H. pubescens*. The probability of internal spread has been made progressively less likely by action of the Dominion Seeds Act administration. By 1926 without knowledge probably of any Canadian infestations of Russian knapweed, the potential menace of the seeds in commercial samples was recognized by the naming of the weed in the Seeds Act. By 1932 it had been moved into a list of Secondary

Noxious Weeds, and by 1936 into Class 1 of Prohibited Noxious Weeds. Recognition of the sinister part being played by imported alfalfa seed might have been possible still earlier, even if not in advance of the danger, had adequate weed surveys, still short of adequate, been provided.

Since 1923 the Canadian Weed Survey of the Division of Botany, the resources of which have been drawn upon in the preparation of this paper, has functioned to provide a reservoir of information regarding the weed flora of Canada. As rapidly as possible data already secured in the field are being put in shape for greater use. Only when that has been done will there be informed direction for the supervised campaign effort against specific weeds, which would seem to be essential to their effectual restriction.

SUMMARY

The history of Turkestan alfalfa in the United States and Canada, and the extent of its functioning as a vehicle of weed infestation are reviewed. Russian knapweed, long known to be an indicator of the Turkestan source of alfalfa seed, is only one of a number of impurities found in samples, and as it now appears, of weeds established, chiefly in the West.

Through the detection of joint occurrence of two or more of these weeds, and particularly of their occasional association with Russian knapweed, certain weeds of south Russian distribution were definitely traced to this imported seed.

The period of unrestricted importation of Turkestan alfalfa seed came to a close for reasons other than those which might have been stressed here, but the greater use of seed testing data and of the results of weed surveys is stressed in order that such widespread invasion shall not be possible again.

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THE EFFECT OF PHOSPHATIC FERTILIZERS ON COMMON ROOTROT[1]

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Among the diverse methods used for controlling plant diseases the use of fertilizers is of importance. In dealing with rootrots, however, reports as to the suitability of various fertilizers for this purpose have been rather indefinite except in the case of browning rootrot. As early as 1923, Dosdall (2) reported the results of field plot experiments designed to show the effect of various fertilizers on infection in wheat and barley from *Helminthosporium sativum* Pammel, King and Bakke. Her results showed no correlation between the amount of the disease and any particular fertilizer. Greaney (3) conducted a series of experiments in which he studied the effect of phosphorus on the infection of wheat seedlings by *Fusarium culmorum* (W. G. Smith) Sacc. in sand cultures in the greenhouse. He concluded that deficiencies of phosphorus failed to influence the severity of the disease, while an excess of phosphorus appeared to accentuate it. Broadfoot and Tyner (1), using *H. sativum* as the parasite, concluded from their laboratory experiments that extremely small concentrations of phosphorus had no effect on the disease, and excess phosphorus produced no significant reduction in the amount of disease. Hynes (4), working in Australia, tested the effect of superphosphate on rootrot arising from artificial inoculation with *H. sativum*. His experiments were conducted out-of-doors in pots and in field plots. The results of his pot experiments indicated that superphosphate produced no beneficial effects. The results of his field experiments in three years out of four indicated the same thing. In the fourth year there was slightly better emergence in the fertilized rows but the disease development in the adult plant stage was not reduced by the fertilizer.

For several years the authors have investigated the influence of phosphatic fertilizers upon the amount of common rootrot (*H. sativum* and *Fusarium* spp.) arising from these fungi occurring naturally in the soil under field conditions. It is a well established fact that triple superphosphate or ammonium phosphate commonly produce marked increases in the yield of wheat on summerfallowed land in Saskatchewan. It is known also, that phosphatic fertilizers often produce such increases in the presence of browning rootrot (6). It was the purpose of these investigations to find out whether or not phosphatic fertilizers cause any reduction in common rootrot in the fertilized wheat.

EXPERIMENTAL METHODS

After some preliminary field plot tests, work was conducted in 1937, 1938, and 1939 according to the following plan. Plots of wheat consisting of 5 rows $18\frac{1}{2}$ feet in length and 6 inches apart were used. These were replicated and randomized in accordance with modern field plot technique.

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One buffer row was sown between adjacent plots, and at the end of the ranges the plots were protected by several border rows. Before harvesting the wheat, one foot was trimmed off the ends of each row, leaving it one rod in length.

The phosphatic fertilizers employed were the ones in common use in Saskatchewan, namely triple superphosphate and ammonium phosphate in 1937 to 1939, and also ammoniated superphosphate in 1939. Triple superphosphate contains 43% phosphoric acid equivalent. Ammonium phosphate is 11% nitrogen and contains 48% phosphoric acid equivalent as well. Ammoniated phosphate is 2% nitrogen and contains 19% phosphoric acid equivalent. These fertilizers were sown into the drill rows with the seed. The ammonium phosphate was applied at the rate of 30 pounds per acre and the other fertilizers at the rate of 33 pounds per acre.

These plots were sown in four or five localities in Saskatchewan each year. These different localities are characterized by different soil types. When the wheat matured the centre 3 rows of each plot were harvested and threshed to determine the relative yields. At the same time the stubble from 100 plants in each plot was examined for common rootrot lesions, and disease rates were computed from the data obtained. In addition to the above mentioned data, in 1937 and 1938 a comparison was made of the relative development of fertilized and unfertilized wheat in the seedling stage.

The disease ratings were based on the lesions appearing on the subcrown internodes of the plants. The plants were grouped into three classes depending on whether the lesions were slight, moderate or severe. A fourth class consisted of plants free from lesions of the subcrown internodes. It had been found by the junior author (5) that the average yields of plants with lesions of the subcrown internodes were reduced as compared with the yields of plants free from such lesions. The number of plants in each class was multiplied by factors based on their relative yields of grain, as follows: severe lesion group, 4; moderate group, 2; and slight group, 1. Summing the three products, dividing by 10 and converting to a basis of 100 plants gives the disease rating. This method of arriving at a rating of the disease is an attempt to measure the actual losses in yield. A number of factors, however, make any close approximation of the losses uncertain. It is felt, nevertheless, that for purposes of comparison of treatments, under any one set of conditions, the method is reasonably reliable.

EXPERIMENTAL RESULTS

The 1937 data on the relative development of fertilized and unfertilized wheat in the seedling stage are based on 100 unfertilized seedlings and 100 seedlings fertilized with triple superphosphate at each place. The material was taken from extra plots put in for that purpose. The data obtained are presented in Table 1.

The figures in the table show differences in favour of the fertilized seedlings at each place. The differences were greater at some places than at others.

TABLE 1.—DATA CONCERNING THE RELATIVE DEVELOPMENT OF FERTILIZED AND UNFERTILIZED WHEAT SEEDLINGS IN 1937

Place	Treatment	Height, (cm.)	No. of tillers	No. of leaves	No. of crown roots	No. of seminal roots
Muenster	Triple superphosphate	38.1	3.0	11.5	5.9	5.5
	Check	36.1	2.7	10.4	3.4	5.0
Prudhomme	Triple superphosphate	43.3	4.2	17.8	7.6	5.0
	Check	37.7	3.7	15.1	6.5	4.4
Rosthern	Triple superphosphate	29.5	3.9	9.6	3.3	6.4
	Check	27.8	3.4	8.3	2.1	6.1
Saskatoon	Triple superphosphate	33.4	4.3	12.6	3.4	6.3
	Check	29.4	3.6	11.1	3.1	5.4

In this area wheat suffered considerably from drought in 1937. The plots at Saskatoon were too badly stunted to harvest the grain, but disease counts were taken on the stubble. At Rosthern the wheat was badly stunted in patches, at Prudhomme it was uniformly stunted but produced a fair yield of grain, while at Muenster it produced a normal crop.

TABLE 2.—AVERAGE YIELDS AND DISEASE RATINGS IN 1937

Treatment	Yields in grams per plot			
	Muenster	Prudhomme	Rosthern	Saskatoon
Control	239	217	130	—
Triple superphosphate	417	245	125	—
Ammonium phosphate	353	244	127	—
	Disease ratings			
Control	11.1	16.5	28.8	23.1
Triple superphosphate	12.8	18.0	30.7	25.4
Ammonium phosphate	12.2	16.4	29.8	24.9

TABLE 3.—ANALYSES OF VARIANCE OF YIELDS AND DISEASE RATINGS IN 1937

Source of variance	Degrees of freedom	Mean squares for yields*			
		Muenster	Prudhomme	Rosthern	Saskatoon
Replicates	3	1564	594	3956	—
Treatments	2	**32517**	983	22	—
Error	6	622	216	1056	—
		Mean squares for disease ratings*			
Replicates	3	5.59	0.86	0.63	0.94
Treatments	2	3.01	3.00	3.53	5.64
Error	6	4.26	1.46	1.80	1.82

* Mean squares in boldface type exceed the 1% level of significance.

The yields and disease ratings of the wheat are shown in Table 2, and the analyses of variance of yields and disease ratings are given in Table 3. The phosphatic fertilizers produced no significant differences in yields at Rosthern but they increased the yields at Prudhomme and Muenster. The disease ratings of the fertilized wheat were somewhat higher, in nearly every case, than those of the unfertilized wheat, but the differences were not statistically significant.

In 1938 similar plots were laid out at Aberdeen, Muenster, Prudhomme, Rosthern and Saskatoon. The comparative development of the fertilized and unfertilized wheat in the seedling stage is indicated by the data in Table 4. The relative height and number of tillers is a good measure of the degree of stimulation at this stage of development. The response to the fertilizers was marked at Muenster and Prudhomme, but at Rosthern there was little or no difference. Other data gathered at several different places in other years have shown the same general tendencies in seedling development in fertilizer tests, as are shown in Tables 1 and 4. In most cases the superior development of the fertilized seedlings was apparent to the eye.

TABLE 4.—DATA CONCERNING THE RELATIVE DEVELOPMENT OF FERTILIZED AND UNFERTILIZED SEEDLINGS IN 1938

Treatment	Height in centimeters				
	Aberdeen	Muenster	Prudhomme	Rosthern	Saskatoon
Check	41.2	42.4	35.3	49.5	37.7
Triple superphosphate	45.8	54.8	44.0	50.8	39.6
Ammonium phosphate	46.8	56.5	43.3	50.7	43.1
	Tillers per plant				
Check	3.1	1.6	1.3	3.2	2.2
Triple superphosphate	3.8	2.4	2.4	3.9	2.8
Ammonium phosphate	3.4	2.3	2.5	3.2	2.7

As in 1937, conditions were too dry to produce a high yield of grain. The results obtained are shown in Table 5, and analyses of variance of these data are shown in Table 6. As may be seen from these tables, yields were not secured at Muenster and Rosthern. The crop on the plots at Muenster was partially destroyed by livestock and that at Rosthern was dried out. Disease rates, however, were computed from data obtained from the stubble collected at each location. The fertilized plots yielded more grain than the checks at Prudhomme and Saskatoon but the differences in yields at Saskatoon were not statistically significant. There were no differences in yields at Aberdeen. The differences in disease rates shown by check and fertilized plots were highly significant only at Prudhomme and Rosthern.

In 1939 ammoniated phosphate was added to the list of fertilizers, and one set of the plots was sown at Scott instead of at Rosthern. The Saskatoon plot was ruined, for experimental purposes, by uneven germination caused by insufficient moisture in the soil in May. The results obtained at the other four places are shown in Table 7, and the analyses of variance in Table 8.

TABLE 5.—AVERAGE YIELDS AND DISEASE RATINGS IN 1938

Treatment	Yields in grams per plot				
	Aberdeen	Muenster	Prudhomme	Rosthern	Saskatoon
Control	288	—	157	—	163
Triple superphosphate	285	—	240	—	187
Ammonium phosphate	290	—	212	—	182
	Disease ratings				
Control	25.0	12.2	11.7	21.6	8.0
Triple superphosphate	26.2	13.7	16.2	24.6	8.9
Ammonium phosphate	25.7	11.1	20.1	24.4	9.1

TABLE 6.—ANALYSES OF VARIANCE OF YIELDS AND DISEASE RATINGS IN 1938

Source of variance	Degrees of freedom	Mean squares for yields*				
		Aberdeen	Muenster	Prudhomme	Rosthern	Saskatoon
Replicates	5	**5028**	—	*7293*	—	2859
Treatments	2	40	—	*10874*	—	967
Error	10	861	—	1629	—	906
		Mean squares for disease ratings*				
Replicates	5	*16.47*	**27.27**	7.93	4.22	9.28
Treatments	2	2.05	*10.65*	**106.43**	**16.65**	1.82
Error	10	3.55	2.53	4.00	1.82	4.80

* Mean squares in boldface type and italics exceed the 1% and 5% levels of significance respectively.

TABLE 7.—AVERAGE YIELDS AND DISEASE RATINGS IN 1939

Treatment	Yields in grams per plot			
	Aberdeen	Muenster	Prudhomme	Scott
Control	777	546	602	299
Triple superphosphate	879	791	581	280
Ammonium phosphate	877	758	593	279
Ammoniated superphosphate	856	654	593	293
	Disease ratings			
Control	11.5	5.8	25.2	26.7
Triple superphosphate	17.5	9.3	27.0	27.9
Ammonium phosphate	18.9	11.5	28.8	26.4
Ammoniated superphosphate	15.8	9.5	27.4	27.5

TABLE 8.—ANALYSES OF VARIANCE OF YIELDS AND DISEASE RATINGS IN 1939

Source of variance	Degrees of freedom†	Mean squares for yields*			
		Aberdeen	Muenster	Prudhomme	Scott
Replicates	5	1784	**5677**	**14952**	1432
Treatments	3	**13902**	**73776**	445	763
Error	15	949	909	1030	857
		Mean squares for disease ratings*			
Replicates	5	7.92	5.51	*6.29*	**43.12**
Treatments	3	**61.09**	**34.76**	**13.36**	3.78
Error	15	2.92	2.51	1.84	2.49

† For Scott the degrees of freedom are, replicates 7, treatments 3 and error 21.
* Mean squares in boldface type and italics exceed the 1% and 5% levels of significance respectively.

As may be seen from the yields recorded in Table 7, the wheat developed well and produced a high yield at Aberdeen, Muenster and Prudhomme. At Scott, however, conditions were very dry and the yields were only moderately good. No significant differences in yields resulted from the use of the phosphates at Prudhomme and Scott. At Aberdeen moderate and significant differences in yield were obtained, and at Muenster large and highly significant differences developed. At both of the latter places ammoniated superphosphate produced smaller increases in yield than did the stronger fertilizers. The disease rates from the fertilized plots were significantly greater than those from the check plots at all places except Scott where there were no significant differences.

DISCUSSION

The results presented show that there are a number of factors which complicate the study of the effect of phosphatic fertilizers on rootrots under field conditions. One of these is the variable response of the wheat plants to such fertilizers in different years in the same field. At Prudhomme in 1937 the wheat which received applications of phosphate produced an average increase in yields of grain over the unfertilized wheat of about 13%, and in 1938 the increase was nearly 44%. On the other hand in 1939, although there occurred a noticeable stimulation in the seedling stage, there was a decrease of about 2% in yields of grain at harvest time. A similar situation as to yields arose at Scott in 1939. The fertilized wheat produced lower yields than the unfertilized, whereas, in most seasons, phosphatic fertilizers cause marked increases in yield in the Scott district.

Failure to secure an increase in yield, on soil where increases usually occur, may be due to either of two different sets of conditions. In the one case the soil may be too dry throughout the early development of the wheat to dissolve the fertilizer so that the wheat seedlings can use it. In the other case the fertilizer may produce a much heavier growth in the early part of the season and subsequent drought conditions may affect the fertilized wheat more severely than it affects the unfertilized wheat. The latter situation appeared to occur to a limited degree at Prudhomme in 1939.

In some cases the application of phosphatic fertilizers produces no visible effect upon the growth of the wheat. At Aberdeen in 1938 and at Rosthern in 1937 there were no significant differences in either yields or disease rates. These results suggest that the amount of phosphorus available in the unfertilized plots was sufficient for the normal growth of the wheat under the particular conditions prevailing at those places in the years mentioned.

The data presented suggest that the application of phosphatic fertilizers is more likely to affect the severity of common rootrot when other conditions are such that the yields are influenced. The analysis of variance of yields and disease ratings show that significant increases in yield were usually accompanied by significant increases in disease rating. Instances of this may be seen in the results obtained at Prudhomme in 1938 and at Aberdeen and Muenster in 1939. Conversely, where there were no significant increases in yield there seldom occurred significant differences in disease ratings. The results seem to show a correlation, in any one test, between the more vigorous growth of the fertilized wheat, where the wheat responded to the application of phosphates, and the higher disease rates.

These experiments fail to show any diminution in the severity of common rootrot through the application of phosphatic fertilizers. On this point at least, these results agree with those of the other investigators mentioned above. Moreover, in our tests the fertilized wheat exhibited somewhat higher disease rates than the unfertilized wheat in nearly every case. Notwithstanding this fact, the fertilized wheat frequently yielded more grain even though it showed a higher disease rate. Apparently the increase in yields, due to the phosphates, more than balanced the losses due to the increase in disease. Under other conditions it is possible that the influence of the disease might outweigh the influence of the phosphatic fertilizers.

SUMMARY

The effect of phosphatic fertilizers upon the severity of common rootrot of wheat was studied under natural field conditions.

Fertilized wheat usually exhibited a somewhat higher disease rate than unfertilized wheat. Nevertheless, the fertilized wheat frequently produced higher yields than unfertilized wheat in the presence of common rootrot.

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